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# **PREVENTION OF CERVICAL CANCER IN COUNTRIES WITH HIGH AND LOW INCIDENCE OF THE DISEASE**

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# Prevention of Cervical Cancer in Countries with High and Low Incidence of the Disease

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*To my daughters*



## ABSTRACT

Cervical cancer is to a large extent a preventable disease, through prophylactic human papillomavirus (HPV) vaccination and cervical cancer screening. Still, it is the second most common cancer among women in Argentina, with unchanged mortality rates for the last 30 years, due to the absence of organized screening. In Sweden, with a well-organized screening program, cervical cancer burden is lower; however, the incidence is increasing again. This thesis has explored several aspects of cervical cancer prevention in different settings. The overall aim of this thesis is to improve cervical cancer prevention by contributing knowledge on i) vaccination acceptance, ii) barriers to screening and iii) the risk of recurrent/residual disease among women treated for precancerous lesions.

Study I explored HPV vaccination acceptance among 174 young women aged 18-30 years from the Mendoza Province, Argentina, by using a structured questionnaire-based interview technique. HPV vaccination acceptance was at high 95%, and 75% stated that they were also willing to pay for vaccination. A statistically significant positive association was found between acceptance and belief in vaccine safety, and a statistically significant negative association between being a welfare recipient and acceptance if vaccination was not free. Educational campaigns ensuring the safety of vaccines, as well as clarifying other misconceptions are needed.

Study II investigated maternal HPV vaccination acceptance among 180 mothers to girls aged 9-15 years from the Mendoza province, Argentina, through use of a structured survey. HPV vaccination acceptance was 90% if it was free of charge, and 60% if it was not free. Being gainfully employed and having a higher disposable household income was significantly associated with acceptance of vaccination if it was not free, which suggests that cost could be an obstacle to catch-up vaccination and may lead to socioeconomic inequalities in uptake. Also, women with prior awareness of cervical cancer were more willing to pay for HPV vaccination, indicating the importance of improving awareness of HPV and its related diseases.

Study III explored barriers and facilitators of screening compliance among 1510 women attending cervical cancer screening in Stockholm, Sweden through use of a structured survey. The mean total time and travel costs and direct non-medical cost per attendance were €55.6. Nearly half (44%) of the women did not attend screening within 1 year from their invitation, of which 51% cited difficulties in taking time off work. The most important correlates of higher screening compliance were not needing to take time off work, not having a companion and being of higher HPV knowledge. Increased flexibility by extended opening hours and improved general knowledge of HPV may facilitate screening compliance.

Study IV investigated the long-term risk of recurrent or residual disease in relation to surgical margin status among 991 women who had undergone conization treatment of high-grade precancerous lesions at the Karolinska University Hospital in Sweden during 2000-2007. During a median of 10 years follow-up, 12% were diagnosed with residual or recurrent disease, based on data from the Swedish National Cervical Screening Registry. Women with involved margins had significantly worse outcomes compared to women with negative margins. The risk was almost 3-fold higher among women with involved endocervical margins, involved margins and uncertain as to whether these were endocervical or ectocervical, and especially when both margins were involved. These findings suggest that stratified margin status may contribute to the safety of the follow-up surveillance-

In conclusion; this thesis provides improved knowledge on what may constitute barriers to HPV vaccination and cervical cancer screening, and delineates possible strategies on how to increase uptake of these prevention strategies. Ensuring the safety of HPV vaccines and educational campaigns on HPV and its related diseases appear important targets for achieving higher uptake. Also, our findings suggest that costs can be a barrier, both in the Argentinean setting, as well as to participation in cervical cancer screening in Sweden. It is thus important that the screening organization facilitates the participation of all women in Sweden, including all socioeconomic groups. Finally, the thesis shows that women treated for precancerous lesions with involved surgical margins are at high-risk of recurrent disease and differential risks could be shown with respect to which margin was affected. Future research should address the accuracy of combining margins status with established follow-up surveillance.



## LIST OF SCIENTIFIC PAPERS

- I. ALDER S, Perinetti C, Mints M, Belkić K, Sundström K, Sandin S, Weiderpass E, Andersson S  
**Acceptance of human papillomavirus (HPV) vaccination among young women in a country with a high prevalence of HPV infection.**  
*Int J Oncol. 2013 Oct;43(4):1310-8.*
- II. ALDER S, Gustafsson S, Perinetti C, Mints M, Sundström K, Andersson S.  
**Mothers' acceptance of human papillomavirus (HPV) vaccination for daughters in a country with a high prevalence of HPV.**  
*Oncol Rep. 2015 May;33(5):2521-8.*
- III. Östensson E, ALDER S, Elfström KM, Sundström K, Zethraeus N, Arbyn M, Andersson S.  
**Barriers to and facilitators of compliance with clinic-based cervical cancer screening: population-based cohort study of women aged 23-60 years.**  
*PLoS One. 2015 May 26;10(5):e0128270*
- IV. ALDER S, Megessi D, Sundström K, Östensson E, Mints M, Andersson S.  
**Incomplete excision of cervical intraepithelial neoplasia as a predictor of the risk of recurrent disease – a 16 year follow-up study.**  
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## LIST OF ABBREVIATIONS

ADC	Adenocarcinoma
AGC-neoplastic	Atypical glandular cells, suspicious for AIS or cancer
AGC-NOS	Atypical glandular cells not otherwise specified
AIS	Adenocarcinoma in situ
AJCC	The American Joint Committee on Cancer
ANOVA	Analysis of variance
ART	Antiretroviral therapy
ASC-H	Atypical squamous cells cannot exclude HSIL
ASC-US	Atypical squamous cells of undetermined significance
CDC	Centers for Disease Control and Prevention
CI	Confidence Interval
CIN	Cervical intraepithelial neoplasia
CIN2+	CIN2 or worse
CIS	Carcinoma in situ
C-LETZ	Contoured loop excision of the transformation zone
CO2 laser	Carbon dioxide laser
CRPS	Complex Regional Pain Syndrome
CT	Computed tomography
DPT	Diphtheria, Pertussis and Tetanus
EFC	The European Federation of Colposcopy
EMA	The European Medicines Agency
EUR	Euro
FDA	The US Food and Drug Administration
FIGO	The International Federation of Gynecology and Obstetrics
GACVS	WHO Global Advisory Committee on Vaccine Safety
HC2	Hybrid Capture2
HIV	Human immunodeficiency virus
HLA	Human leukocyte antigen
HPV	Human papillomavirus
HR	Hazard Ratio

HR-HPV	High-risk HPV
HSIL	High-grade squamous intraepithelial lesions
IARC	The International Agency for Research on Cancer
ICC	Invasive cervical cancer
ICD	International Classifications of Diseases
LBC	Liquid-based cytology
LEEP	Loop electrosurgical excisional procedure
LLETZ	Large loop excision of the transformation zone
LR-HPV	Low-risk HPV
LSIL	Low-grade squamous intraepithelial lesions
MEB	Department of Medical Epidemiology and Biostatistics
MMR	Measles, Mumps, and Rubella
MRI	Magnetic resonance imaging
MSM	Men who have sex with men
OR	Odds Ratio
PCR	Polymerase chain reaction
PET	Positron emission tomography
POTS	Postural Orthostatic Tachycardia Syndrome
pRb	Retinoblastoma protein
RCT	Randomized controlled trial
RR	Relative Risk
SEK	Swedish kronor
SCC	Squamous cell carcinoma
SCJ	Squamocolumnar junction
STI	Sexually transmitted infection
TBS	The Bethesda system
TNM	The tumor, node and metastasis system
TZ	Transformation zone
VAIN	Vaginal intraepithelial neoplasia
VLP	Virus-like particles
WHO	The World Health Organization

# 1 INTRODUCTION

Noble Prize winner Harald Zur Hausen made the first association between Human papillomavirus (HPV) and cervical cancer in 1976, which has dramatically changed the cervical cancer prevention field. Nowadays it is well established that HPV is necessary for cervical cancer development. This knowledge has led to several scientific breakthroughs such as the introduction of prophylactic HPV vaccines in 2006 and the more recent large-scale use of HPV tests to screen for cervical cancer as well as a test-of-cure during follow-up after treatment. The likewise important use of organized cervical cancer screening has dramatically reduced the burden of cervical cancer in many parts of the world. Despite these progresses within the field and that it to a large extent is a preventable disease, cervical cancer is the fourth most common cancer among women globally. There are still many obstacles to overcome, such as global inequalities in access to cervical cancer screening and HPV vaccination. Today cervical cancer prevention is often lacking in regions where it is needed the most. Also, in regions with well-established screening and vaccination programs, an important issue is to achieve high uptakes of screening and vaccination.

Despite Argentina being an upper middle-income country, cervical cancer incidence is similar to figures seen in low-income countries. The country is lacking a well-functioning screening program and HPV vaccination may be the most beneficial way to reduce cervical cancer burden over time. Organized school-based HPV vaccination was introduced in Argentina in 2011, although data on vaccination acceptance in connection with this was scarce. In contrast, Sweden has a relatively low cervical cancer incidence and mortality due to the well-organized screening program. However, for not fully understood reasons, the incidence has now started to increase again. Largely, there are two major risk-groups of developing cervical cancer in Sweden; women who do not attend screening and those previously treated for precancerous cervical lesions.

The final goal of this thesis is to contribute to cervical cancer prevention, both in countries with high and low burden of disease. There are different targets for improving cervical cancer prevention, and their relative importance differs between various settings of the world. Therefore, this thesis aims to i) explore HPV vaccination acceptance in Argentina, ii) identify barriers of cervical cancer screening in Sweden and iii) investigate the long-term risk of residual or recurrent disease among women previously treated for precancerous lesions in Sweden, and to assess this risk in relation to surgical margin status. Thereby, we hope to contribute with knowledge on strategies on how to increase vaccination and screening uptakes as well as on the risk-stratification of women treated for precancerous lesions.





## 2 BACKGROUND

### 2.1 CERVICAL CANCER

#### 2.1.1 The cervix

The cervix is the lowest part of the uterus and is mostly composed of fibro-muscular tissue. It is subdivided into the endocervix and the ectocervix: the distal ectocervix (portio) protrudes into the vagina and is visible at pelvic examination using a speculum, while the proximal endocervix extends towards the uterus and is not visible. The cervical canal goes through the cervix and leads to the uterine cavity. The ectocervix is lined with a multi-layered squamous epithelium and the endocervix, including the endocervical canal, is lined with a single layer of columnar epithelium. The transition area between these two types of epithelium is called the squamocolumnar junction (SCJ). Over time, a physiological process takes place, in which the fragile columnar epithelium is exposed to the acidic environment from the vagina, which results in replacement of the columnar epithelium with squamous epithelium, creating a new SCJ. This process is called metaplasia (1). The SCJ thus migrates thru life and goes from being fully visible at the surface of the portio in adolescence into the endocervical canal in post-menopausal women (2). The region between the old and new SCJ is called the transformation zone (TZ). For unknown reasons, this zone is especially susceptible to HPV infections and, consequently, most squamous cervical cancers arise from the TZ (Figure 1) (1). There are two main types of cervical cancer that originates from the two types of cervical epithelium: squamous cell carcinoma (SCC) and adenocarcinoma (ADC), where SCC by far is the most common (84%) (3).

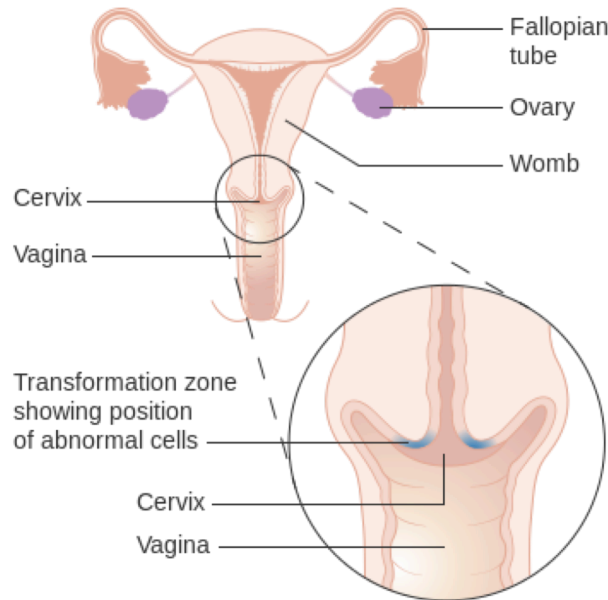
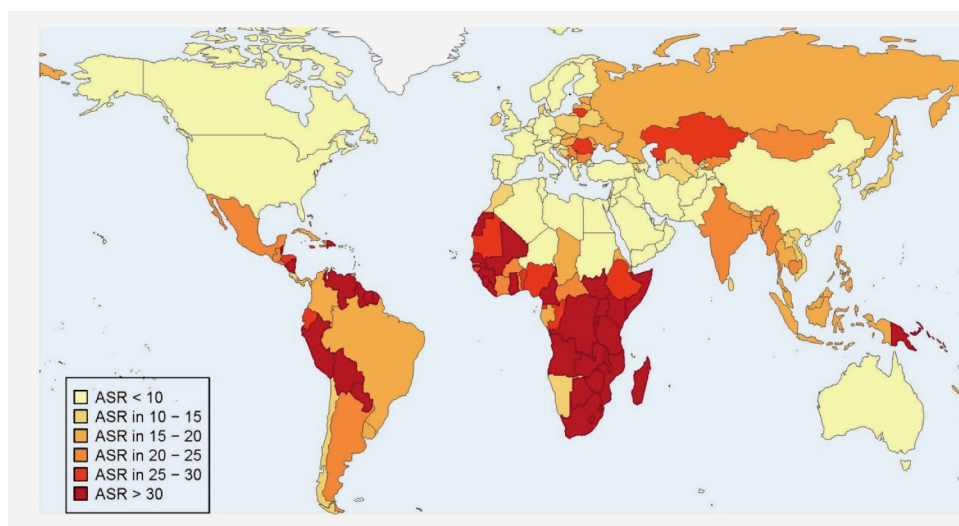


Figure 1. The anatomy of the cervix and the transformation zone. This image has been released as part of an open knowledge project by Cancer Research UK. If re-used, attribute to Cancer Research UK / Wikimedia Commons;

[https://commons.wikimedia.org/wiki/File:Diagram\\_showing\\_the\\_transformation\\_zone\\_on\\_the\\_cervix\\_CRUK\\_375.svg](https://commons.wikimedia.org/wiki/File:Diagram_showing_the_transformation_zone_on_the_cervix_CRUK_375.svg).

### 2.1.2 Cervical cancer epidemiology

Cervical cancer is the fourth most common cancer among women worldwide, after breast, colorectal and lung cancer, with an estimated 528,000 new cases and 266,000 deaths in 2012 (4). In contrast to most other malignancies, almost half of the new cases are seen in women under the age of 50 (5). The worldwide distribution of cervical cancer varies greatly, with 84% of all cases and 87% of deaths occurring in less developed regions. Cervical cancer remains the second most common cancer in less developed regions, while nowadays it is less common (11<sup>th</sup>) in more developed regions. The overall age-standardized cervical cancer incidence is 14 per 100,000, although ranging from 4.4 to 42.7 per 100,000. High-burden areas with an overall age-standardized incidence over 30 per 100,000 are Eastern, Southern and Middle Africa and Melanesia. In some of these regions—Eastern and Middle Africa—cervical cancer is the predominant female cancer (4). Besides the above-mentioned regions, individual countries with an age-standardized incidence over 30 per 100,000 are also found in Latin America (5). In contrast, Australia/New Zealand and Western Asia represent the regions with the lowest incidence worldwide (Figure 2). The overall age-standardized cervical cancer mortality is 6.8 per 100,000 ranging from the lowest: 1.5 per 100,000 in Australia/New Zealand to the highest 27.6 per 100,000 in Eastern Africa (4).



*Figure 2. Age-standardized (world) incidence rates (per 100,000) of cervical cancer cases attributable to HPV in 2012. © 2017 International Agency for Research on Cancer (IARC/WHO); licensed by UICC, Reproduced with kind permission from de Martel et al (5).*

### 2.1.3 Human papillomavirus and the etiology of cervical cancer

It is now widely accepted that the sexually-transmitted HPV has a causative role in the development of cervical cancer. Retrospective studies have detected oncogenic high-risk HPV (HR-HPV) in almost 100% of all cervical cancers cases (6, 7). HPV is a large and heterogeneous family of viruses, with around 150 identified types infecting humans (8), of which around 30-40 infect the anogenital tract (9). Of these, 13 types are recognized as oncogenic to humans: HPV 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59 and 68. Twelve of these HR-HPV types have been classified by the International Agency for Research on Cancer (IARC) as carcinogenic to the cervix and type 68 is classed as probably carcinogenic (10). HPV 16 is identified as the most potent HPV type in cervical carcinogenesis, followed by HPV 18 (10). HPV 6 and 11 cause condyloma (genital warts), but are not carcinogenetic, and are thus classified as low-risk HPV (LR-HPV) (10). A meta-analysis on the worldwide distribution of HPV types in 30,000 invasive cervical cancer (ICC) cases, showed that HPV 16 was the most frequent HPV type in ICC in all regions of the world (57%, 95% Confidence Interval (CI), 54.3-58.9), followed by HPV 18 (16%, 95% CI, 14.6-17.4). Together they account for about 73% of all ICC. The next most common HPV types were HPV 58, 33, 45, 31, 52, 35, 59, 39, 51 and 56 in descending order, although their relative order varied in different regions of the world. The distribution of HPV also differed between ADC and SCC. HPV 18 was most frequent in ADC (36.8%, 96% CI 34.9-39.7), while HPV 16 was most common in SCC (59.3, 95% CI 56.8-61.7) (11).

#### 2.1.3.1 HPV-related cancer outside the cervix

It has been estimated that 2.2 million cancers worldwide, representing 15% of all cancers are caused by infectious agents. Around 640,000 of these cases were attributable to HPV, which makes HPV the second most common infectious agent in cancer worldwide, after *Helicobacter pylori* (which causes gastric cancer) (12). HPV has been estimated to cause 4.5% of all cancer worldwide, 8.6% of all cancers in women and 0.8% of all cancers in men (5). IARC has classified HPV 16 as not only causing cervical cancer, but that sufficient evidence exists of its carcinogenicity in vaginal, vulvar, penile, anal, oral, oropharyngeal and tonsillar cancer. HPV 18 and 33 have also been associated with carcinogenesis outside the cervix (10). It has been estimated that 78% of all vaginal, 25% of all vulvar, 90% of all anal, 50% of all penile and 30% of all oropharyngeal cancer is attributed to HPV (5).

#### 2.1.3.2 HPV transmission and prevalence

HPV is the most common sexually transmitted infection (STI) worldwide, and most people will be infected with HPV at some point during their lifetime (10). Asymptomatic HPV infections are common in both men and women; however, HPV causes more disease in women due to the vulnerability of the cervix to HPV infection (13). The transmission rate seems to be higher for female-to-male transmission, with studies reporting a 19-81% cumulative incidence over a 6-month period, compared to a rate of 5-28% for male-to-female transmission. It has also been reported that auto-inoculation from cervix to anus, or reversed, in the same individual is relatively common (14). A case-control study including over 5,000 patients with head and neck cancer and 6,000 controls found that history of oral sex was significantly associated with cancer of the base of the tongue, oropharynx, and tonsil, suggesting a genital-oral transmission of HPV, as well (15).

A large meta-analysis on over one million women has shown an overall prevalence of 12% in women with normal cytology. The highest estimates were found in Sub-Saharan Africa (24%), Eastern Europe (21%) and Latin America (16%). The most common HPV types globally are: HPV 16, 18, 52, 31, 58, 39, 51, and 56, where HPV 16 predominates in almost

all regions, while the subsequent order of HPV types varies by region. HPV infection is most common among women below age 25 and declines with increasing age, although in many continents an unexplained second peak in HPV prevalence can be observed for middle-aged women (16). However, local very high prevalence of HPV has been reported. For example, a study on youth health clinic attendees in Stockholm found a notably high HR-HPV prevalence of 62% among this “risk-group”, of which 35% were HPV 16 positive and 61% were infected with more than one HR-HPV type (17). For men, reliable data on genital HPV prevalence is scarce, although it is estimated to be around 50%, although with a higher proportion of LR-HPV and lower rates of persistence (18) (14). Contrary to HPV infections in women, there is no clear age-distribution and HPV prevalence seems rather steady across age groups in men (18).

#### 2.1.3.3 HPV taxonomy and organization of HPV genome

Papillomaviruses are small, double-stranded, non-enveloped ancient DNA viruses, which can be dated back several 100 million years. As previously mentioned, it is a large and diverse group and new papillomaviruses and HPVs are detected continuously. With few exceptions, HPVs mainly infect the skin, oral-, anal- and genital- squamous mucosal epithelia, as well as the endocervical columnar epithelia, but not epithelia in other locations (19). The HPVs are classified into Alpha-, Beta-, Gamma-, Mu- and Nu- papillomavirus based on their phylogenetic relationship, epithelial tropism, and diseases that they cause. The Alpha papillomaviruses can infect the mucosa and skin, while the others infect only the skin. Thus, the Alpha papilloma group includes LR-HPV which causes condyloma, as well as the 13 previously mentioned HR-HPVs causing cervical cancer (8).

The HPV genome consists of a control region and eight genes. The early genes E1, E2, E4, E5, E6, and E7 are important for the viral cell cycle, while the late genes L1 and L2 encode the capsid proteins (Figure 3) (8). E1 and E2 are involved in viral replication and are necessary for the initial amplification phase, while E5, E6, and E7 are important for the host cell environment, as the viral DNA replication uses the host cells replication system (8, 20). There are large differences between LR-HPV and HR-HPV regarding E6 and E7 protein functions, as well as promoter regulation (8).

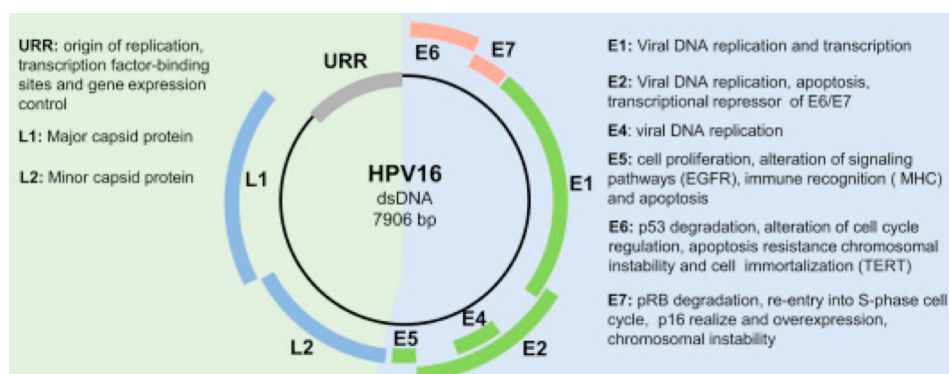


Figure 3. HPV 16 structure and viral proteins. Reproduced with kind permission from de Sanjose et al. (13).

#### 2.1.3.4 The HPV life-cycle

The squamous epithelium of the cervix is multi-layered and as the epithelia self-renews, new daughter cells migrate upwards after cell division. It is believed that the HPV enters the epithelium in the susceptible TZ through a micro-wound and accesses the basal layers of the

epithelium, that serve as a reservoir for the infection. Due to the natural cell division of the basal cells, new HPV-infected daughter cells are produced and migrate upward (8). When the epithelial cells differentiate, a large up-regulation of HPV gene expression takes place, including E6 and E7, as well as the late capsid genes (9). This results in complete virions being released at the surface with potential of transmission to others (Figure 4) (9, 13). It has been reported that HR-HPV, in contrast to LR-HPV, more often infects basal cells that still possess proliferative properties and also activates cell-proliferation to a greater extent in these cells (13).

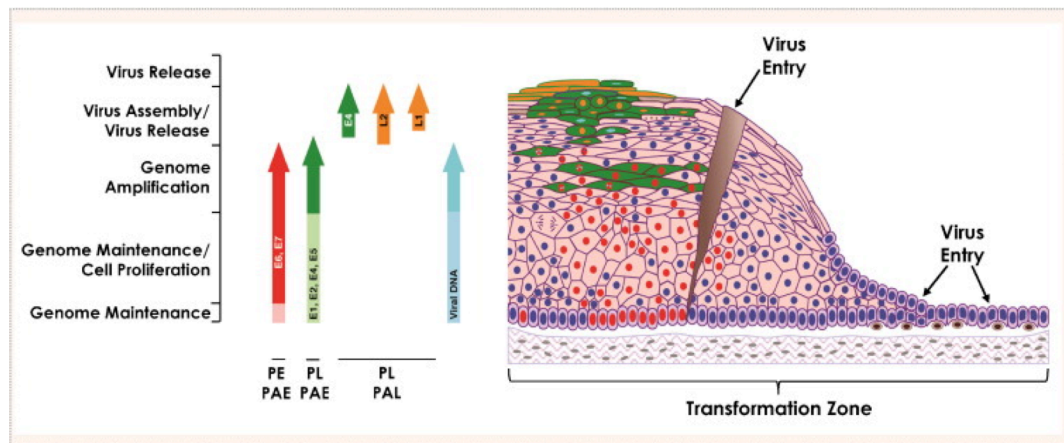


Figure 4. Life Cycle of High-Risk HPVs in Cervical Epithelium. Reproduced with kind permission from Doorbar et al. (8).

#### 2.1.3.5 Immune response to HPV and HPV clearance

The viral life cycle is exclusively intraepithelial, without viremia, cell death, or inflammation, making it difficult for the immune system to detect the infection. Consequently, immune evasion is considered to be the hallmark of HPV infection (8, 9). Still, the majority (80-90%) of all HPV infections (including infections by HR-HPV) are transient and cleared by the innate immune system within one to two years (8). Recent studies have also pointed out the role of T-cells from the adaptive immune system in HPV clearance (21). Persistent HPV infection is necessary for the development of high-grade dysplasia or invasive cervical cancer and the risk increases with duration of persistence (22). It has been reported that long-term persistence of HR-HPV, i.e. for seven years or more, without development of cervical intraepithelial neoplasia grade 3 (CIN3) or worse is rare (14). Persistent HPV infection is more common in women above age 30 compared to younger women (22).

It has been discussed whether or not a once cleared HPV infection can re-activate in the same individual. Figures on the frequency of re-detection of HPV after a documented clearing, range between 5-20%. It has been found that re-detection often is related to sexual activity and it has therefore been considered that re-detection is due to re-exposure to HPV. As HPV is cleared by the innate immune system, it does not result in memory cells, and re-infection is possible. However, in some women, antibodies can be detected, which suggests that they have probably had a persistent infection. Among these women, the relation between sexual activity and re-detection of HPV is not as clear, and the possibility of re-activation of a latent infection has not been ruled out completely (14).

#### 2.1.3.6 HPV induced carcinogenesis

The recognized mechanism of HPV carcinogenicity is by the production of oncoproteins E6 and E7, which leads to a down-regulating of tumor suppressor genes p53 and retinoblastoma protein (pRb), that normally regulate uncontrolled or abnormal growth and protect against cancer. E7 binds to and degrades pRb, which controls cell cycle entry, while E6 indirectly mediates the degradation of p53. Further, E6 and E7 can inactivate interferon regulatory factor and thereby avoid detection by the immune system and avoid apoptosis (20). E6 has also been found to be capable of up-regulating telomerase activity (8). The expression of E6 and E7 is low in the early stages of infection, but increases over time through mutations and integration of viral DNA (20). It has been shown that the levels of E6 and E7 increase from CIN1 to CIN3. With further accumulation of genetic errors, these cells can progress to invasive cancer (8). It seems that E5 also contributes to carcinogenesis, although with a less significant role and mainly in the beginning of infection. E5 has been reported to stimulate cell proliferation and prevent apoptosis of DNA damaged cells (23).

### 2.1.4 Co-factors and surrogate markers of HPV infection

The single most important risk factor for cervical cancer is persistent HR-HPV infection; still only a few infected women will develop ICC. Other risk factors of cervical cancer, besides HPV, are now classified as either surrogate markers for risk of HPV infection or as co-factors among HR-HPV infected women (24).

#### 2.1.4.1 Sexual behavior and other STI

Total number of sexual partners increases the risk of HPV exposure and hence cervical cancer. A lifetime number of 6 or more partners versus 1 partner is associated with a more than twofold risk of ICC (2.78, 95% CI: 2.22-3.47) and 11 or more partners with a threefold risk (3.15, 95% CI: 2.19-4.52). Early age at first intercourse has also been associated with an increased risk of ICC, also after adjusting for total number of sexual partners. Suggested explanations for this is that early sexual debut would serve as a marker of high-risk sexual behavior i.e. exposure to HPV, or that early intercourse could result in a longer lifetime exposure to HPV or that the cervix of young women is more susceptible to HPV infections (25).

Co-infection with other STIs has been considered as a potential co-factor of cervical cancer, although results are conflicting and confounding effects of HPV have been suggested. A large study on half a million women found no association of herpes simplex type 2 and cervical cancer, when adjusted for HPV status and smoking (26). Neither did a large prospective study, in which biobank samples from women who had developed cervical cancer were analyzed regarding STIs (27). On the other hand, more evidence exists on a true association between Chlamydia trachomatis and cervical cancer. In the same prospective study, a statistically significant association between Chlamydia trachomatis and cervical cancer was found, also when restricting the analysis to HPV positive women (Odds Ratio (OR): 1.4, 95% CI: 1.1–2.0) (27). This association was confirmed in a recent meta-analysis, which showed a fourfold increased risk of cervical cancer among women with concomitant HPV and Chlamydia trachomatis infection (OR: 4.03, 95% CI: 3.15-5.16) (28). The cause of this is not known, although it has been reported that Chlamydia Trachomatis increases HPV persistence, possibly through chronic inflammation (29).



#### *2.1.4.2 Hormonal contraceptives and high-parity*

Hormonal contraceptives and high-parity have also been discussed as co-factors of cervical cancer. After the finding of the important role of HPV in carcinogenesis, the significance of these associations has been questioned. Some studies restricted to women with HPV positive findings have shown a statistically significant increased risk of CIN3 and cervical cancer among women with over 5 years use of oral contraceptives (30, 31), while others have not (32). However, most studies support a significant association between oral contraceptives and cervical cancer, and that the risk increases with duration. Therefore, it has been classified as carcinogenic to the cervix by the IARC (33). A recent prospective study on more than 300,000 women confirmed previous findings on oral contraceptive use, also after adjusting for HPV. Oral contraceptive was significantly associated with increased risk of cervical cancer and increased with duration of contraceptive use (Hazard Ratio (HR): 1.8 for oral contraceptive use  $\geq 15$  years compared to never use). In the same study, it was found that increasing number of full-term pregnancies was positively associated with CIN3; however, no significant association with cervical cancer was found (34). This association was, however, seen in a study on women with HPV positive findings, where the number of full-term pregnancies increased the risk of cervical cancer compared to nulliparous women (OR 2.3, 95% CI: 1.6-3.2 for 1 to 2 full-term pregnancies and OR 3.8, 95% CI: 2.7-5.5 for 7 or more full-term pregnancies). Neither the association between high-parity nor oral contraceptives in cervical carcinogenesis is fully understood. However, it seems that oral contraceptives interact with hormone receptors and influence HPV expression of E6 and E7 oncogenes, rather than making women more susceptible to HPV infection (33, 34).

#### *2.1.4.3 Smoking*

Smoking is identified as carcinogenic to the cervix by the IARC and considered an important co-factor for cervical cancer (35). In an analysis restricted to HPV positive women, a twofold risk of SCC was seen among smokers compared to never smokers (Relative Risk (RR): 1.95, 95% CI 1.43-2.65) (36). In a more recent study on 300,000 women, a similar two-fold risk of either high-grade squamous intraepithelial lesions (HSIL) or ICC was seen among smokers compared to never smokers, after HPV adjustment. Smoking cessation reduced the risk (37). Smokers have been described as having more mutations in their cervical mucus and also a higher frequency of DNA adducts than non-smokers, which could implicate these mechanisms in cervical carcinogenesis (35).

#### *2.1.4.4 Immunosuppression*

Another important co-factor is immunosuppression. A meta-analysis on patients with Human immunodeficiency virus (HIV) and patients receiving immunosuppressive treatment due to transplantation found that both groups were more prone to develop HPV-related cancers as well as other infection-related cancers (38). Women with HIV-positive findings have been found to have a two to three-fold increased risk of acquiring HPV and lower rates of HPV clearance (RR 2.64, 95% CI: 2.04-3.42 and HR 0.72, 95% CI 0.62-0.84, respectively) compared to women who had HIV-negative findings. Also, the risk of cervical cancer is four-fold higher in HIV-positive versus HIV-negative women (RR 4.1, 95% CI 2.3-6.6). The risk of acquiring HPV was higher in patients with low CD4 counts and lower in women on antiretroviral therapy (ART) (39). The importance of ART to reduce HPV related disease burden in HIV-positive women was confirmed in another meta-analysis, which showed lower risk of CIN2 or worse (OR 0.59, 95% CI, 0.40-0.87) as well as cervical cancer (HR 0.40, 95% CI 0.18-0.87) among patients on ART (40).

#### 2.1.4.5 Genetic predisposition

Genetic predisposition has also been suggested as a co-factor for cervical cancer, since only a small percentage of women infected with HR-HPV develop CIN or cervical cancer (8). A registry-based study has shown an increased risk of cervical cancer among women with a first-degree or second-degree relative with cervical cancer, although this could be influenced by environmental as well as heritable factors (41). Most HPV infections are cleared by the immune system, such that host-factors related to this cell-mediated clearing could be important as to why some women develop HPV related disease. Absence of, or limited T-cell response to HPV antigens has been reported in women with cervical cancer (8). Human leukocyte antigen (HLA) is involved in antigen presentation and the association between variations of HLA and cervical cancer have been investigated in numerous studies. However, the evidence supporting this association is inconclusive (42).

### 2.1.5 Precancerous lesions of the cervix

#### 2.1.5.1 Classification of precancerous lesions

Cervical cancer develops over time and is preceded by precancerous lesions of various grades (43). There are different classification systems in use for grading the severity of these lesions. The system by Richart was introduced in 1966 and is based on histopathological findings (43). It grades precancerous lesions into cervical intraepithelial neoplasia, CIN, stages 1 to 3 on the basis of the amount of abnormal cells and their appearance. CIN1 represents mild dysplasia, CIN2 moderate dysplasia and CIN3 severe dysplasia with atypical cells throughout the entire epithelium, equivalent to carcinoma in situ (CIS). Invasive cervical cancer, ICC, is defined by abnormal cells penetrating the epithelial basement membrane (Figure 5) (44).

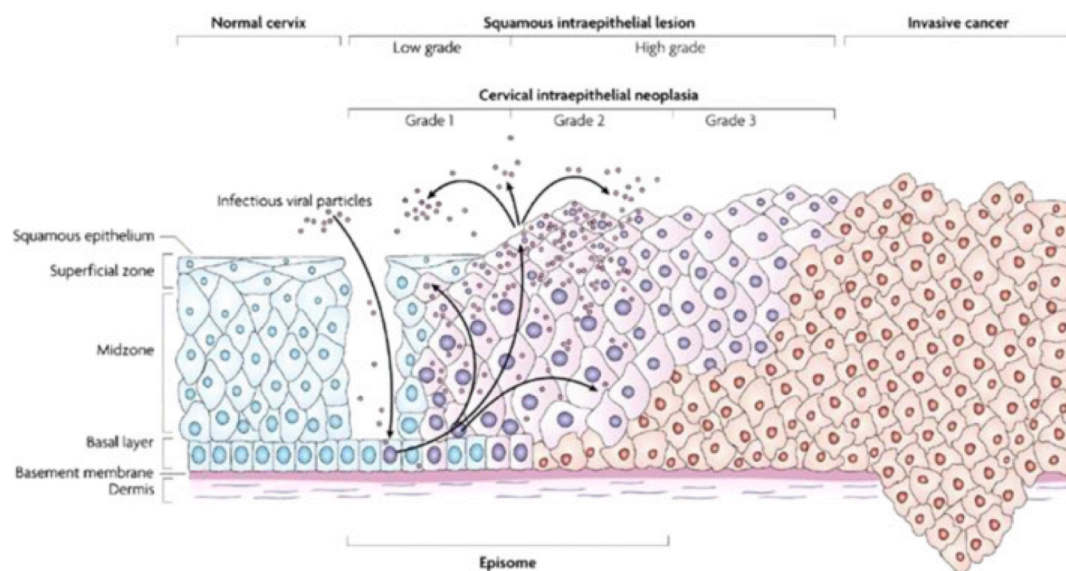


Figure 5. Schematic representation of the HPV infection in cervical mucosa and its different potential in squamous intraepithelial lesions. Reproduced with kind permission from de Sanjose et al. (13).



Due to the various terminology in use and inter-observer variability in grading lesions, a uniform classification system, the Bethesda system (TBS), was introduced in 1988, based on cytology (45). The nomenclature has been revised throughout the years, and current classifications include low-grade squamous intraepithelial lesions (LSIL) equivalent to CIN1 and high-grade squamous intraepithelial lesions, HSIL, equivalent to CIN2/3. In addition, the Bethesda system has a nomenclature for atypical squamous cells of undetermined significance (ASC-US) and atypical squamous cells cannot exclude HSIL (ASC-H). Also, atypical glandular cells are sub-classed as atypical glandular cells not otherwise specified (AGC-NOS), atypical glandular cells, suspicious for AIS or cancer (AGC-neoplastic) and adenocarcinoma in situ (AIS) (Table 1) (46).

New Swedish national cervical cancer guidelines from Jan 12, 2017 recommend a shift from the CIN-nomenclature to TBS for classification of cytological abnormalities (47), in line with European recommendations (48). The national recommendations include that also histopathological dysplasia should be classified as either LSIL or HSIL on the basis of international consensus (47, 49). For women aged 27 years or below, it is, however, still recommended to divide HSIL into CIN2 or CIN3 due to the high regression rate of CIN2 in this age-group (50). Young women with CIN2 could thus be kept under surveillance to avoid over-treatment (49).

Table 1. Conversion table for different cytological classification systems. Reproduced with kind permission from Herbert et al. (51)

Papanicolaou	WHO	CIN10	TBS 19912	TBS 20015
I	Normal			Negative for epithelial abnormality
II	Atypia		Infection, reactive repair	
			ASCUS	ASC-US
				ASC-H
	Atypical glandular cells		AGUS	Atypical glandular cells
III	Mild dysplasia	Condyloma	LSIL	LSIL
		CIN I		
	Moderate dysplasia	CIN II	HSIL	HSIL
IV	Severe dysplasia	CIN III		
	CIS			
	AIS	CGIN	AGUS	AIS
V	Invasive carcinoma			

#### 2.1.5.2 *Natural history of precancerous lesions*

The natural history of precancerous lesions has been studied retrospectively during times when cervical lesions were managed conservatively. A Canadian study showed that most CIN1 and CIN2 would regress spontaneously, rather than progress. The cumulative percentage of progression from CIN1 to CIN3 or worse was 2% at 2 years, 6% at 5 years, and 10% at 10 years. Corresponding figures for CIN2 to CIN3 or worse was 16% at 2 years, 25% at 5 years and 32% at 10 years (52). Reports on the risk of progression of CIN3 to cervical cancer have, for understandable reasons, been scarce. However, a long-term follow-up on an unethical clinical study from the 1960s, in which women were withheld treatment for CIN3, showed that the cumulative incidence of cervical or vaginal cancer was 31% within 30 years. In a subgroup of women with persistent CIN3 after 2 years, half (50%) of the women progressed to invasive disease by 30 years. In contrast, the corresponding cumulative incidence in women receiving adequate treatment was 0.7% (53).

The regression rates have been reported to be higher in younger women. In a study on mainly younger women with a median age of 23 years, the regression rate of CIN2 was approximately 40% (54). The natural history of precancerous lesions is also influenced by HPV type. It has been found that women infected with HPV 16/18/45 are diagnosed with SCC at a younger age compared to women infected by other HPV types. This suggests a more rapid progression to invasive cancer through infection with any of these three HPV-types (55).

#### 2.1.6 **Invasive cervical cancer**

Cervical cancer is mainly spread by local invasion in the pelvis, but can also spread to regional lymph nodes or metastasize to lung, liver, bone and brain (56). Early stages of cervical cancer can be asymptomatic, while abnormal bleeding is a cardinal symptom (e.g. intermenstrual, post-coital and postmenopausal bleeding). Other symptoms include persistent vaginal discharge or in more advanced stages pain, e.g. due to compression of nerves, or symptoms from the rectum or bladder due to tumor spread to these organs (1, 57).

After a histopathologically-confirmed diagnosis of cervical cancer, staging is performed, including tumor size, whether it has invaded adjacent organs or has spread to distant locations. There are different staging systems in use; staging according to the International Federation of Gynecology and Obstetrics (FIGO) system is a clinical evaluation, which has been useful for low-resource settings (58). FIGO-staging can also be performed by surgical staging, which has been found to be more accurate than clinical staging and is standard in many high-resource settings (59). Stage I is cancer that does not extend outside the cervix; subdivided into stage IA-IB2, with stage IA being microinvasive cancer and IB2 with lesions of 4 cm or larger. Stage II extends outside the cervix, up to the upper two thirds of the vagina, but does not involve the pelvic wall; stage III involves the pelvic wall or the lower one third of the vagina. Stage IV is disseminated disease, either to adjacent pelvic organs (urine bladder and/or rectum, stage IVA) or metastasized to distant organs (stage IVB) (60, 61). Another staging system is The American Joint Committee on Cancer (AJCC) Tumor, node, and metastasis (TNM) system, which additionally takes the lymph node status and pre-treatment radiologic examinations into account using computed tomography (CT), magnetic resonance imaging (MRI) and positron emission tomography (PET) scan (62). Survival rates are related directly to stage and the 5-year survival rates decline with increasing stage, from 76-98% in stage I, 66-73% in stage II; 40-42% for stage III to 9-22% for stage IV (63). However, lymph node invasion is another important prognostic factor of survival (64). Therefore, TNM staging has been suggested to be more accurate in assessment of prognosis (62).

Treatment is also based on staging, and early stages are mainly treated by surgery. Surgical procedures include fertility-preserving treatment with conization or trachelectomy for the earliest stages or simple hysterectomy or radical hysterectomy. Lymph node dissection is performed on all women, except those with stage 1A1 and no lymphovascular space invasion. Radiotherapy or chemotherapy can be used as adjuvant treatment depending on surgical findings and stage or to be used alone for later stages of disease (58).

The global 5-year survival rate for women with cervical cancer has been estimated based on registry data on 660,000 women. The 5-year survival for women diagnosed 2010-2014 was found to be 60–69% in most countries. Japan, Korea, Taiwan, Denmark, Norway, Switzerland and Cuba were the only countries with an estimated 5-year survival rate of 70% or higher, while 13 countries were in the lower range between 50-59%, located in South and Central America, Asia and also some European countries. These global variations are presumably due to differences in stage at time of diagnosis and inequalities in treatment (65).

## **2.2 PREVENTION BY CERVICAL CANCER SCREENING**

### **2.2.1 Cervical cancer screening**

The purpose of cervical cancer screening is to detect precancerous lesions and to treat them and thereby reduce cervical cancer incidence and mortality (66). The great impact of organized screening in reducing cervical cancer burden has, among others, been shown in several Swedish studies, in which a decline by approximately two-thirds has been observed (67-69). As mentioned above, the majority of all cervical cancer cases occur in less developed regions of the world (4), largely due to lack of or ineffective screening programs in these regions (70). Indeed, cervical cancer incidence in more developed regions was, before the introduction of organized screening, comparable to figures seen in less-developed regions today (71). The greatest impact of cervical cancer screening is seen on reducing SCC, while limited reduction has been shown for ADC (67-69). More developed countries have a higher proportion of ADC, and about one-fifth of all cervical cancer cases in these regions are ADC (3). In contrast to SCC, an increase of ADC has been seen in several European countries despite screening. A possible reason for this could be changes in sexual behavior with an increasing number of women being infected with HR-HPV, while at the same time the screening program is less effective for detecting ADC (72).

#### *2.2.1.1 Screening by conventional cytology*

The conventional cytology, i.e. the Pap smear from the 1940s, named after its developer Dr. George Papanicolaou, remains the screening standard method in many countries (73). Cervical cells are sampled from both the ectocervix and endocervix, then smeared onto a glass slide and fixated, often with 95% ethyl alcohol, and finally microscopically examined. European guidelines recommend the use of a spatula to collect material from the ectocervix in combination with an endocervical brush or using a cervical broom or an extended tip spatula alone. It is important that the sample includes material from the TZ, since, as described above, most dysplasia arises from this area (74). A systematic review has shown that the sensitivity and specificity for Pap smears to detect cytological abnormalities varies greatly, with figures ranging from 30-87% for sensitivity and 86-100% for specificity (75). Despite the often low sensitivity of Pap smear, its use has effectively reduced cervical cancer incidence, presumably due to the repeat testing within screening programs (73).

### *2.2.1.2 Liquid-based cytology*

Due to the low accuracy of conventional cytology and poor quality caused by obscuring factors (e.g. blood), the liquid-based cytology (LBC) technique was developed in the late 1990s (76). Cervical cells are collected in the same way for LBC as for conventional cytology, but rinsed and preserved in liquid before being transferred to a microscope slide. Methods in use are ThinPrep (Cytoc, Boxborough, MA, USA) and SurePath system (TriPath Imaging Inc., Burlington, NC, USA) (74). It has been reported that LBC results in more uniform samples and less obscuring factors, and could thereby facilitate cytological assessments. Importantly, the method also facilitates supplementary testing, including reflex HPV testing. However, there is no evidence that cervical dysplasia is better detected by LBC than conventional cytology (73). A randomized controlled trial (RCT) on almost 90,000 women did not show any statistically significant differences in detection rates between the two methods (76). Still, many of more developed countries have shifted to LBC (73). It is also the method in use for cytological diagnostics in Sweden (47).

### *2.2.1.3 Primary HPV testing in cervical cancer screening*

The major discovery of the causal role of HPV infections in cervical cancer has spurred new approaches for cervical cancer screening using HPV testing. Evidence from four RCTs has found HPV-based screening to be more sensitive than conventional screening for detecting CIN3 (77-80). In 2014, Ronco and colleagues presented results from a follow-up study on these four RCTs, and were able to show a 60-70% greater protection also for ICC with HPV-based screening compared to conventional screening. The study also found that the cumulative incidence of ICC was lower 5 years after a negative HPV test than 3 years after negative cytology. The authors recommended a shift to HPV-based screening for women over 30 years with a 5-year interval (81). The advantage of the HPV tests is thus the high longitudinal negative predictive value, i.e. very little risk of developing cervical cancer following a negative HPV test, which permits prolonged screening-intervals. This is very important also from a cost perspective (73). The follow-up study could not show any benefits in reducing ICC using HPV-based screening in women under the age of 30 (81). Also, in the previous study by Ronco et al. it was described that HPV-based screening leads to an over-diagnosis of CIN2 in women aged 24-35, as CIN2 to a large extent would regress spontaneously in this age-group (77). This together led to recommendations that HPV-based screening should begin from the age of 30 (81).

European guidelines on cervical cancer screening from 2015 now recommend primary HR-HPV testing in the organized cervical cancer screening programs. It is also recommended that primary HPV screening should not begin under the age of 30 and that the screening interval should be at least 5 years for women with a negative primary HPV test, in agreement with results from the RCTs (82). These recommendations have also been implemented in Swedish guidelines (47).

There are numerous different HPV tests on the market that detect viral DNA or RNA. HPV-DNA is mainly detected by either hybridization with signal amplification, or by genomic amplification, typically using polymerase chain reaction (PCR) (73). Two HPV-DNA tests, Hybrid Capture2 (HC2), QIAGEN and GP5+/6+ PCR were used in most RCTs that compared HPV-based screening to cytology screening and detect all thirteen HR-HPV types (82). The reported pooled sensitivity of these two tests is 96.1 (95% CI, 94.2%-97.4%) and a specificity of 90.7% (95% CI, 90.4%-91.1%) (83). Due to the rapid development of different HPV assays, a guideline for HPV-DNA tests requirements has been developed. The recommendations include that the sensitivity should be not less than 90% of the sensitivity of Hybrid Capture2 and specificity no less than 98%, of the specificity of Hybrid Capture 2 and

have an intra-laboratory reproducibility of at least 87% (84).

#### *2.2.1.4 HPV self-sampling*

Another advantage of the HPV test is that it can be performed by self-sampling. In a meta-analysis, it was found that the sensitivity to detect CIN2 or worse for HPV self-sampling was 76% (95% CI, 69–82%) and the specificity to exclude CIN2 or worse was 86% (83–89%). This was significantly lower than HPV test performed by a clinician. Still, the test can have an important value, especially to be sent home to screening non-attendees, in order to increase adherence to screening (85). In a Swedish study, HPV self-sampling kits were delivered to 8,000 women who had not participated in screening for 6 years or more. The overall response rate was 39% and among those women who had an HR-HPV positive result, the majority (89%) went to follow-up examination. Almost one in four HPV positive women (23%) was found to have CIN2 or worse during follow-up (86). This emphasizes the importance of targeting this high-risk group.

### **2.2.2 Follow-up colposcopy**

Women identified with HR-HPV positivity or cytological abnormalities through the national screening program are managed and followed-up according to standardized procedures (87). In Sweden, among women aged 23-29 years where cytology is the primary screening modality, a reflex HPV test is conducted on women with ASCUS or LSIL, while women with HSIL and ASC-H are referred to colposcopy without an HPV-reflex test. For women over the age of 30 years, a cytology reflex test is conducted on HR-HPV positive women – and women with cytological abnormalities are referred to colposcopy irrespective of the grade (47).

Colposcopy is a procedure in which the cervix is examined with a colposcope, an instrument that illuminates and magnifies the cervix. It is used to detect visual abnormalities and to guide biopsy for histological assessment if there are abnormal findings. During the colposcopy, the lesion size, lesion borders, surface contours, the vascular pattern, acetowhite changes and iodine uptake are observed. If dilute (3–5%) acetic acid is applied to the cervix, abnormal cells turn white. Acetowhite changes that last for one minute have an increased risk of being dysplasia. Similarly, applying Lugol iodine solution turns abnormal cells bright yellow (1, 88). Various findings on the accuracy of colposcopy to detect precancerous lesions have been reported. A meta-analysis found the sensitivity to detect all cervical abnormalities ranging between 87–99%, while the specificity was lower (23–87%) (89). A more recent meta-analysis from 2016 found sensitivity and specificity estimates to be more diverse, ranging between 29-100% and 12-88%, respectively (90). Due to the low accuracy of colposcopy, different scoring systems for colposcopy assessments have been evaluated. The Swedescore has shown 95% specificity with a score of 8 points or more for CIN 2 or worse (90). The Swedescore are therefore used to assess colposcopy in Sweden (47).

### **2.2.3 Treatment of precancerous lesions**

Due to the risk of progression of precancerous lesions to ICC, the guidelines from The American Society for Colposcopy and Cervical Pathology and the common practice is to treat women with histopathological CIN2 or worse (CIN2+). Younger women with CIN2 could, however, be kept under surveillance (87). Women under 25 years have a high incidence of CIN2 and high recurrence rate, while the cervical cancer incidence is low (91). CIN3, on the

other hand, should be treated regardless of age due to the higher risk of progression to ICC. CIN1 should be managed by observation, although persistent CIN1 should be treated, or closely followed-up if preceded by HSIL cytology. Treatment of CIN1 in younger women should be avoided (87). Swedish practices are in accordance with these guidelines (47). Treatment is mostly performed by excisional treatment (conization) where a cone-shaped portion of the cervix is removed or by non-excisional treatments. Common treatment modalities are loop excision of the transformation zone (i.e. LEEP (loop electrosurgical excisional procedure) in the US and LLETZ (large loop excision of the transformation zone) in the UK), laser conization, cryotherapy, cold knife conization and laser ablation. A Cochrane review on the different surgical treatment options found an overall success rate of around 90%. There was no evidence regarding the superiority of any treatment technique. Cryotherapy was mainly recommended for treatment of low-grade disease and was considered a good option for low resource settings due to low costs (92). Women who have been treated for high-grade dysplasia have an increased risk of recurrent CIN, cervical and vaginal cancer for at least 10-25 years after treatment, and it is therefore important that these women are followed adequately (93-96). The risk of recurrence remains increased for at least 10-25 years post treatment (94-96). A recent meta-analysis found an overall recurrence/residual rate of 6.6% among women treated for CIN (97). Women with involved margins in the cone biopsy have been reported to have a higher risk of recurrent disease (97, 98). This is also the case for women with HPV persistence during follow-up. Co-testing with HPV test and cytology as a test-of-cure is now supported by international guidelines and existing evidence (87, 99).

#### *2.2.3.1 Side effects of treatment*

There are perioperative and postoperative complications related to treatment of precancerous lesions and overtreatment should be avoided. Reported short-term complications include bleeding, infections or damage to nearby organs. A meta-analysis from 2016 compared complications related to LEEP, cryotherapy and cold knife conization, and found adverse events to occur in less than 1% of women who were treated, although bleeding was more common with cold knife conization (2.4%). It was, however, noted that the studies often were of low quality and that more studies assessing the frequency of complications were needed (100). Several meta-analyses have investigated the risk of adverse pregnancy outcomes after treatment of CIN. In a 2017 meta-analysis by Kyrgiou et al. (101), treatment was associated with an almost twofold risk of premature birth (<37 weeks) (RR 1.75, 95% CI, 1.57 -1.96). The risk was highest after cold knife conization, (RR 2.70, 95% CI, 2.14-3.40) followed by laser conization (RR 2.11, 95% CI, 1.26-3.54) and somewhat lower for LLETZ (RR 1.58, 95%, CI 1.37-1.81). Women with several treatments had an almost four-fold increased risk compared to women without previous treatment (RR 3.78, 95% CI, 2.65-5.39). It was also found that the risk increased with greater depth of the surgical specimen from a RR of 1.54 (95% CI 1.09-2.18) with a depth of 10-12 mm or less, to a RR of 4.91 (95% CI 2.06-11.68) with a depth of 20 mm or more. It should, however, be noted that women with CIN had a higher baseline risk of premature birth compared to the general population which indicates that the results could also be influenced by other confounding factors (101).

## **2.2.4 Barriers and health economic aspects of screening**

### *2.2.4.1 Health economic aspects*

Health economics represents an interdisciplinary method to assess the value of health policy decisions, taken in complex settings, to improve healthcare. Since resources are scarce, health economics is concerned with issues relating to efficient allocation of given resources to

maximize health outcomes (102). According to the guidelines by the World Health Organization (WHO) and the Swedish National Board of Health and Welfare [Socialstyrelsen], health policymakers need to ascertain both the clinical effectiveness and cost-effectiveness of any population-based screening program (103, 104). Given that compliance with cervical cancer screening is crucial to both these factors, eliminating potential barriers is especially important.

Women who do not participate in cervical cancer screening have an increased risk of cervical cancer (66). Cervical cancer prevention can thus be optimized further by increased screening coverage also in countries with already well-established cervical cancer screening programs (105). The screening uptake in the member states of the European Union has been found to vary between 10-79% and Sweden represented the country with the highest uptake (106). However, the screening uptake has been reported to have declined in younger women in many developed countries (107). A study from Sweden found an association between young age and lower participation rate. Other factors associated with lower participation were being single and not being in the labor force (108). Another Swedish study found immigrant women to have a lower participation rate than Swedish-born (49% to 62%) (109). This was, however, not shown in a large population-based case-control study on 600,000 Swedish women, when adjusting for socioeconomic factors. The study did show a strong association between socioeconomic status and screening participation. Important factors associated with lower screening participation were low disposable family income (OR 2.06; 95% CI, 2.01-2.11), low education (OR 1.77, CI 1.73-1.81) and not cohabiting (OR 1.47, CI 1.45-1.50) (110). A meta-analysis on qualitative research addressed how women's experiences and perceptions influenced screening uptake. Two major themes were identified: questioning the relevance and the value of participation and screening posing a threat or previous negative experiences (111). Strategies discussed to increase adherence to screening could be through use of invitations and reminders. Another promising option to increase screening adherence could be use of HPV self-sampling among non-attenders, as described above (105).

A screening program in general has to be cost-effective on a large scale (103). However, also cost incurred by the patient is important to consider. A study that investigated participation in mammography screening among low-income women found a higher participation rate among women who randomly were given a voucher for a free mammogram compared to those who had to pay the fee. The main reason for non-participation among women without a voucher was cost, while it was transportation for those with a voucher (112). A meta-analysis on strategies to improve cancer screening attendance concluded that the most effective interventions were organizational change, followed by patient financial incentives and patient reminders (113). However, there is very little research that relates to indirect costs (e.g. time spent on the screening visit) or direct-non-medical costs of screening (e.g. cost related to transportation, child care or parking).

#### *2.2.4.2 Barriers in developed countries*

As described above, there are large differences in cervical cancer burden between less developed and more developed countries, primarily due to the existence of screening programs. The main barriers to screening implementation in less developed countries are poverty, as well as related problems of lack of healthcare infrastructures and trained personnel needed for the complex screening process (114). Some less developed countries have opportunistic screening, which means that screening is performed if women seek gynecologic care for other reasons. According to the WHO, opportunistic screening programs mainly cover young women at low risk of cervical cancer, while organized screening programs target the risk population more cost-effectively (1). As previously discussed, HPV

tests have a higher longitudinal negative predictive value than conventional screening and screening intervals can be prolonged (73). A 2016 study that estimated the costs for different screening scenarios in less developed regions concluded that a shift to HPV based screening may be cost-beneficial (115).

## **2.3 PREVENTION BY HPV VACCINATION**

### **2.3.1 HPV Vaccines**

#### *2.3.1.1 Development of vaccines*

The development of HPV vaccines has greatly improved the possibility to prevent cervical cancer. Prophylactic HPV vaccines have been available since 2006 and among the first countries to introduce organized HPV vaccination were Australia, Austria, Belgium, Canada, France, Germany, Italy and the US (116). By 2017, HPV vaccine had been implemented to national vaccination programs in at least 71 countries (117), and an estimated 118 million women had been vaccinated in 2014 (118). The two vaccines used throughout the world are the bivalent (Cervarix<sup>®</sup>, GlaxoSmithKline) and the quadrivalent (Gardasil<sup>®</sup>, Merck and Co). Both vaccines protect against HPV16 and 18 (119), which, as mentioned previously, account for around 70% of all ICC (11). The quadrivalent vaccine also protects against HPV types 6 and 11, responsible for causing > 90% of condyloma (120). In 2014, the US Food and Drug Administration (FDA) approved a nonavalent vaccine (Gardasil 9<sup>®</sup>, Merck and Co), targeting a total of 9 HPV types (6, 11, 16, 18, 31, 33, 45, 52, and 58) (119), which together cause 90% of all cervical cancers (5).

#### *2.3.1.2 Immunogenicity and duration*

All three vaccines contain virus-like particles (VLPs) against the included HPV types. The VLPs are empty, non-infectious protein shells, derived from the L1 capsid, that mimic the authentic virus. The mechanism of protection is through humoral immunity by antibodies, mainly IgG (119). The antibody levels induced by vaccination have been reported to be 10-100 times higher than after a natural HPV infection (121). The exact duration of protection conferred by HPV vaccination, and the need for eventual booster doses, is still unclear. Studies have so far shown sustained high antibody levels during 10-years of follow-up, suggesting a maintained protection for decades (122, 123). Also, the 9-valent HPV vaccine has shown sustained protection after 5 years (124, 125), as has the bivalent vaccine, if administered by two-doses, after a median of 7 years (126).

#### *2.3.1.3 Efficacy of HPV vaccines*

The main HPV vaccine trials are FUTURE I (127) and FUTURE II (128) for the quadrivalent vaccine, while PATRICIA (129) and the Costa Rica vaccine trial (130) are the main trials for the bivalent vaccine. All trials have been randomized, double-blind and placebo-controlled. It has been shown that almost 100% of girls and women seroconvert to the included HPV types following immunization with either vaccine (131). A 2011 meta-analysis on RCTs (including FUTURE I and II and PATRICIA) in which more than 44,000 women were included, found both vaccines to be highly efficacious, with 94-95% efficacy against persistent HPV 16/18 infections and 90-96% efficacy against HPV 16/18 related CIN2+ in per-protocol populations of women without prior infection by the included HPV types. The vaccines have also been shown to have a cross-protective efficacy for some phylogenetically related HPV types not



included in the vaccines. The overall cross-protection for HPV 31/33/45/52/58 related persistence or CIN2+ was 28% and 42%, respectively, which suggests even higher protection rates against cervical cancer (132). The cross-protective efficacy of the two vaccines was compared in another meta-analysis, and the quadrivalent vaccine was found to be efficacious against only HPV 31-related outcomes, while the bivalent vaccine was efficacious against outcomes related to HPV 31, 33, and 45 (133). In 2016, a 10 year follow-up study was published on the impact of the quadrivalent vaccine. It was reported that up to 90% of persistent HPV 6/11/16/18 infection, up to 90% of condyloma, up to 45% of LSILs and up to 85% of HSILs had been reduced among those who were vaccinated (134).

There are several ongoing RCTs evaluating the nonavalent vaccine (135). The first to evaluate vaccine efficacy was a double-blind RCT, in which 14,000 women were administered either the quadrivalent or the nonavalent vaccine (136). The final efficacy, immunogenicity and safety evaluation from the study were published in 2017, and showed a 97% efficacy against HPV 31/33/45/52/58 related high-grade cervical, vulval, and vaginal disease. It was also shown that the immune response and antibody levels to HPV 6/11/16/18 were non-inferior compared to those who received the quadrivalent vaccine (125). Studies have also shown that the nonavalent vaccine is safe and efficacious, also for women previously vaccinated with the quadrivalent vaccine (137).

#### *2.3.1.4 Vaccination of different age-groups*

The recognized strategy for HPV vaccination is to target adolescent girls prior to the initiation of sexual activity, as HPV is transmitted through sexual contact. The American Academy of Pediatrics therefore recommends vaccination for girls aged 11–12 and catch-up vaccination for females aged 13–26 years (138). It has also been shown that girls reach higher antibody titers compared to adult women (139, 140), and that the titers remain higher over time (123). HPV vaccines were first administered by three-doses (including the main trials) but recent trials have shown that two doses of the vaccines were non-inferior in girls/young women (141, 142). In 2014, the WHO therefore revised their recommendations to a two-dose regimen for HPV vaccination for girls aged 15 years or younger (119). This change will result in both cost- and logistic benefits and is projected to be especially important for low-income countries (142). Also, the nonavalent HPV vaccine has been shown to generate non-inferior immunogenicity when administered by 2-doses (143). The 2-dose regimen is now approved by the FDA also for the nonavalent vaccine (144).

The immunogenicity of HPV vaccines in older women has been studied on women up to age 45 years for the quadrivalent vaccine and on women up to age 55 years for the bivalent vaccine (145, 146). Although older women produced lower levels of antibodies compared to younger women (145, 146), both vaccines showed high levels of protection against HPV persistence and CIN1. However, no risk reduction could be shown for CIN2+, which could be due to a low incidence in this age group (145, 147)

#### *2.3.1.5 Vaccination safety*

All three vaccines have shown excellent safety profiles. The meta-analysis on the vaccine trials concluded that there were no statistically significant differences regarding serious adverse events between HPV vaccines and placebo (RR 1.00, 95% CI: 0.91-1.09) nor vaccine-related serious adverse events (RR, 1.82; 95% CI: 0.79-4.20). Mild adverse events such as pain at injection site, headache and fatigue were common (132). The nonavalent vaccine has also found to be safe, although with a higher occurrence of adverse events related to the injection site (most commonly pain, swelling, erythema, and pruritus) compared to the

quadrivalent vaccine (91% and 85%, respectively). This was expected, due to a higher amount of VLPs and adjuvant dose used for the nonavalent vaccine (136).

As mentioned above, around 118 million women had been HPV vaccinated by the end of 2014 (118), contributing to the post-licensure surveillance regarding vaccination safety. A large population-based cohort study from Sweden and Denmark, including around 700,000 doses of the quadrivalent vaccine; found no association between HPV vaccine and autoimmune, neurological or venous thromboembolic adverse events (148). A 2017 updated review on vaccination safety concluded that HPV vaccines are safe based on evidence from over 100 publications (117). Complex Regional Pain Syndrome (CRPS) and Postural Orthostatic Tachycardia Syndrome (POTS) have controversially been associated with HPV vaccination, although evidence from systematic reviews, expert analyses by WHO Global Advisory Committee on Vaccine Safety (GACVS) and the European Medicines Agency (EMA) has shown no causal association (117).

#### *2.3.1.6 Male vaccination*

The quadrivalent vaccine has been licensed for males for some time in many countries (119) and the bivalent and the nonavalent is now also approved for use in boys and men (119, 149). Recent data has shown that anal and HPV-related oropharyngeal cancer is increasing in developed countries in both sexes (119, 150). It is projected that HPV-related oropharyngeal cancer would exceed the number of cervical cancers in the US by the year 2020 (151). Also, men who have sex with men (MSM) have been shown to be a high-risk group for HPV infection and HPV-related cancers, and will not be protected by herd immunity by HPV vaccination of females (152). Therefore, there is an ongoing debate whether to expand HPV vaccination to males, and Australia was the first country to include boys in their vaccination program in 2013. Since then, the US, Austria, Czech Republic, Lichtenstein, Switzerland and Norway have followed (149, 153).

### **2.3.2 Barriers to HPV vaccination implementation**

The introduction of HPV vaccination is key for reducing the global cervical cancer burden, and will have the greatest impact in regions with the highest burden of disease (154). However, it has been reported that only 1% of the 118 million women who had been targeted through national vaccination programs by 2014, were from low-income or lower-middle-income countries. The populations at greatest risk thus remain, to a large extent, unprotected (118). Among the challenges for universal HPV vaccination are high vaccination costs, the maintenance of the cold chain (production, transportation and storing vaccines adequately refrigerated), logistics of implementation of HPV vaccines and vaccination uptake and adherence to the two or three-dose regimen (21, 155). Many low- and middle- income countries with insufficient cervical cancer screening still have well-functioning child vaccination programs (156). The long-term duration of protection of HPV vaccines seen so far, suggests that a feasible option for HPV vaccination implementation could be through such existing programs (21).

#### *2.3.2.1 Uptake and vaccination acceptance*

The most important factor for the impact of vaccination is to attain adequate coverage of the population (156). A study on the impact of HPV 16/18 vaccination on a cohort of 12 year old girls in the UK showed that a theoretical 100% vaccination uptake would reduce cervical cancer incidence and mortality by 76%, but for only 61% if vaccination covered 80% of the

cohort (157). Economic analyses have regarded vaccination coverage of 70% as the threshold for ideal cost-effectiveness (118). Still, many countries have not reached such coverage (21). A recent national immunization survey used by the Centers for Disease Control and Prevention (CDC) in the US showed that only 42% of girl in ages 13 to 17 years had received all doses of the vaccine. A somewhat higher share (63%) had received at least one dose. Still, this was considerably lower in comparison to other childhood vaccines, with corresponding figures for Diphtheria, Pertussis and Tetanus (DPT) and meningococcal vaccine of 86% and 81%, respectively (158). The lower uptake of HPV vaccine in comparison to other vaccines has also been seen in Sweden. Registry data with complete information on vaccinations within the national immunization program from 2016, showed a 2-dose uptake of HPV vaccination of birth cohorts 2000-2004 of 73-77%. At the same time, uptake of DPT and Measles, Mumps, and Rubella (MMR) was above 95% (159). Following the controversial association of POTS and CRPS described above, and other disbelieves in vaccination safety, vaccination uptake in Denmark, Japan and Ireland declined (117).

Parental vaccination acceptance is important to achieve high vaccination uptake (155). A systematic review on parental HPV vaccination acceptance found a wide range of acceptance, between 55 to 100% (160). A similar range was also seen in another meta-analysis on adult women regarding vaccination for self (50-94%) (161). Potential predictors of HPV vaccine acceptance were HPV knowledge, perceived severity of HPV-related disease, perceived effectiveness of vaccines, history of HPV-related disease, self-perception of risk, age, marital status, socioeconomic status, costs and lack of misinformation (160, 161). Two Swedish population-based studies from 2010 investigated parental acceptance and acceptance among young adults, with more than 10,000 respondents each. Acceptance was similar among parents and adult women when vaccination was free (76% and 75%, respectively), while considerably lower for adult women (41%) compared to parents (63%) if vaccination came with a cost. The strongest correlates of acceptance were belief in vaccine safety and in its efficacy. Other positive predictors were prior awareness of HPV, young age, low education, or having someone in the family receiving social welfare (162, 163). Also, the recommendation on vaccination from health care providers has been found critical for vaccination acceptance (164). An RCT on 40,000 participants showed that health interventions could be used to increase HPV vaccination initiation (165). The majority of all studies on HPV vaccination acceptance have been conducted on highly-educated populations, while few investigations have targeted those with low-education, low-income and/or living in rural settings. It has been pointed out that there is a need for studies from low resource settings (166).

## 2.4 CERVICAL CANCER IN DIFFERENT SETTINGS

### 2.4.1 Argentina

#### 2.4.1.1 *Cervical cancer incidence and mortality in Argentina*

Argentina is a high middle-income country, in which cervical cancer remains a major public health problem (167). In 2008 there were an estimated 4,000 new cases and 1,800 cervical cancer related deaths, which makes cervical cancer the third most common female cancer in the country and the second most common cancer among females aged 15-44 years (168). Despite Argentina being a relatively wealthy country, the age-standardized cervical cancer incidence was 20.8 per 100,000 in 2012 (5), which is actually higher than the global average of 14.0 per 100,000 and comparable to figures seen in low-income countries (4). Figures on the age-standardized mortality rate from 2008 were, however, slightly below the global average (7.4 and 7.8 per 100,000, respectively) (168). Still, according to national statistics from the Argentinean government, the mortality has remained almost unchanged, and even increased slightly between 1980 and 2009 (7.1 to 7.5 per 100,000 women) (169). This does not necessarily imply a true increase in mortality, but may rather be the result of improved diagnostic methods and statistics. On the other hand, mortality does not appear to have decreased, even though cytological screening has been practiced for over 50 years (170). However, on a promising note, the 5-year cervical cancer survival rate has been reported to have increased in Argentina from 46% to 51% over a 10 year period between 1995–99 and 2005–09 (171).



Figure 6. Argentina and its provinces.  
[https://commons.wikimedia.org/wiki/File:Argentina\\_-\\_mapa\\_de\\_las\\_provincias.svg](https://commons.wikimedia.org/wiki/File:Argentina_-_mapa_de_las_provincias.svg)

#### 2.4.1.2 *Organization of cervical cancer screening in Argentina*

Cervical cancer screening is mainly opportunistic in Argentina, performed by opportunistic cytology on women seeking health care for other reasons. Before 1998, Argentina did not have a national prevention program for cervical cancer (170). After the implementation of The National Program on Cervical Cancer Prevention, the previous screening recommendations on annual smears from sexual debut to age 64 were revised to the current recommendations on a three-year interval, after two negative annual Pap smears, for women aged 35 to 64 (172). However, the Argentinean health care system is highly decentralized and managed at the provincial level and screening policies vary across the 24 provinces (Figure 6). A study from 2010 investigated the provinces' adherence to the national guidelines. It was found that target age varied between 10-70 years and that screening remained annual in some provinces. Two provinces did not have an official framework for cervical cancer prevention. With few exceptions, there was also an absence of quality control throughout the country (170). A more recent study reported that HR-HPV test now is available in 5 provinces (167).

Overall, there are large geographical inequalities in Argentina, with the age-standardized mortality ranging from a low 3.9 per 100,000 in the capital of Buenos Aires, to 18.9 per 100,000 in the northern Formosa Province (169). There are also socioeconomic inequalities; indeed, the provinces with the highest cervical cancer burden in Argentina are also the poorest provinces (169, 173). Other studies have also confirmed such inequalities, with women of low socioeconomic status being screened less frequently than women of higher socioeconomic status. It has also been reported that these disparities are increasing in provinces with the highest burden of disease (174). Overall, it has been estimated that the current screening practice fails to cover 30% of Argentinean women, while another 40% are screened irregularly (175).

#### *2.4.1.3 HPV vaccination in Argentina*

The HPV vaccine was introduced to the national vaccination program in 2011, with the bivalent vaccine being administered free-of-charge to 11-year old girls (176). There is no subsidized catch-up vaccination, and vaccination for girls and women over the age of 11 is to be self-financed (177). In contrast to the poorly-functioning screening program, Argentina has a well-organized child vaccination program, with an uptake of childhood vaccines between 91-97%, which suggests that implementation of HPV vaccination should be feasible (178). Overall HPV prevalence is higher in South America than the global average (16% versus 12%) (16) and local Argentinean studies have presented even higher figures of 40-47% in the Misiones and Formosa Province (179-181). The HPV 16/18 prevalence in ICC in Argentina is 77% (182), among the highest in the world (183) which suggests a potentially high impact of vaccination. Economic analyses have also found vaccination to be a cost-effective approach (175, 184). Still, there were no studies on vaccination acceptance prior to the implementation of HPV vaccination. At the time of our studies, there was only one study on vaccination acceptance from Argentina, conducted in the Buenos Aires area (185). Thus, there was a particular need for studies from other parts of the country.

### **2.4.2 Sweden**

#### *2.4.2.1 Cervical cancer incidence and mortality in Sweden*

Sweden has had an organized population-based screening program since the 1960s, resulting in a dramatic reduction of cervical cancer incidence and mortality (67-69). The cervical cancer incidence in Sweden has declined by around 70% since the introduction of screening (66). However, the decline has stagnated over the last 10 years (186) and new data even show a 20% increase in the age-standardized cervical cancer incidence in Sweden between 2014 and 2016 (187). The age-standardized incidence and mortality rate of cervical cancer is 8.7 and 1.3 per 100,000, based on 2016 data from the National Board of Health and Welfare. This translates to approximately 540 new cases and 135 deaths each year (186). Cervical cancer is the 10<sup>th</sup> most common cancer among women of all ages in Sweden, but the third most common cancer in the age-group 15-44 years (168). In a nationwide Swedish study from 2008, it was found that two thirds (64%) of women diagnosed with cervical cancer and 83% of women with advanced stages did not attend screening within the recommended screening interval. Non-adherence was found to double the risk of cervical cancer (OR 2.52, 95% CI, 2.19 to 2.91) and quadruple the risk of advanced cancer (OR 4.82, 95% CI, 3.61 to 6.44) compared to attendees (66). Another risk-group for cervical cancer in Sweden is women previously treated for CIN. In another nationwide study, it was shown that women with a history of CIN3 had a two- to six-fold risk of cervical and vaginal cancer compared to the general female population (standardized incidence ratio 2.34 95% CI: 2.18-2.50 and 6.82,

95% CI: 5.61-8.21, respectively). The risk persisted over time and remained increased after 25 years (94).

#### *2.4.2.2 Organization of cervical cancer screening in Sweden*

The Swedish cervical cancer screening program began in 1964 and by 1977 it had nationwide coverage (66). The screening guidelines from the National Board of Health and Welfare have, until recently, recommended cytology screening every third year for women aged 23-50 and every fifth year for women aged 51-60 (188). The screening program invites women to participate by invitation letters and midwives perform the smears at outpatient clinics, although opportunistic screening by gynecologists occurs as well. Both opportunistic and organized smears are registered and integrated into a national screening registry (189). In 2016, the screening coverage was 82% for women aged 23–50 years and 84% for women aged 51–60, although there were large variations among counties. Relatively few women (8%) did not attend screening within a 6 years period (187). In most counties in Sweden, there had been a fee for cervical screening (ranging between 8-22€), while it has been free-of-charge in some counties (189). As of January 2018, cervical cancer screening is free-of-charge throughout the country (190).

As mentioned above, the Swedish screening guidelines have recently been revised. In the new guidelines, primary HPV based screening is to be introduced (and has been introduced in some counties), every third year for women aged 30-49 (although with a supplementary cytology test for women aged 41) and every seventh year for women aged 50-64. Besides the shift to HPV-based screening, the upper age limit has also been raised. For women under the age of 30, cytology screening remains the method of choice (188), in accordance with international recommendations (82). However, it should be noted that the new Swedish screening recommendations differ slightly from the European guidelines in terms of frequency and the supplementary cytology test (82).

#### *2.4.2.3 HPV vaccination in Sweden*

The HPV vaccines were licensed in Sweden in 2006, but the implementation of HPV vaccines in the national school vaccination program was delayed until 2010 due to procurement issues regarding which vaccine should be included. It was decided to use the quadrivalent vaccine, and it is offered free-of-charge for girls aged 11-12 years. Recently, Sweden also shifted to a two-dose regimen of organized HPV vaccination (191). Catch-up vaccination is subsidized for females between ages 13-26, and have been offered free-of-charge in some counties. For girls and women aged 13 years and above, the vaccine is still administered on a three-dose schedule. Boys are, thus far, not included in the national vaccination program and vaccination is not subsidized for males. In July 2017, The Public Health Agency of Sweden [Folkhälsomyndigheten], proposed that boys should also be included in the national vaccination program and is currently under evaluation (153).

## **3 AIMS**

### **3.1 GENERAL AIM**

The overall aim of this thesis is to contribute to cervical cancer prevention in countries with high and low burden of disease, by identifying barriers to HPV vaccination and cervical cancer screening and by exploring the long-term risk of recurrent disease in relation to surgical margin status among women previously treated for precancerous lesions.

### **3.2 SPECIFIC AIMS**

#### **3.2.1 Study I**

To examine acceptance of HPV vaccination among young adult women in Argentina, to explore the correlation of acceptance with cost and other factors, and to study perceptions of HPV vaccination.

#### **3.2.2 Study II**

To investigate maternal HPV vaccination acceptance among Argentinean mothers if vaccination was offered at no cost and if it was not free of charge, to explore correlates of willingness to pay for vaccination, and to study awareness of HPV and its related diseases and attitudes and perceptions of vaccination.

#### **3.2.3 Study III**

To explore barriers and facilitators of screening compliance in Sweden by; estimating time and travel costs and other direct non-medical costs of attending screening, examining screening compliance and correlates thereto, and by investigating knowledge of HPV, its related diseases and cervical cancer prevention and correlates of HPV knowledge.

#### **3.2.4 Study IV**

To investigate the long-term risk of recurrent or residual disease in relation to surgical margin status among women previously treated for high-grade dysplasia in Sweden. Secondary aims were to assess the risk with respect to age, the severity of the lesion and treatment modality.

## 4 MATERIALS AND METHODS

### 4.1 STUDY SETTING, STUDY DESIGN AND STUDY POPULATION

#### 4.1.1 Study I and II

Both studies were hospital-based cross-sectional surveys conducted in the Mendoza province, Argentina. Study I comprised 174 young adult women attending the Obstetrics/Gynecology Ward and the Outpatient Gynecology Clinic of the Diego Paroissien Public Hospital in Maipú, and Study II included 180 mothers attending Diego Paroissien Public Hospital or the Private Italian Hospital in the city of Mendoza. Eligibility criteria required the women in Study I to be between 18 and 30 years of age, while the mothers in Study II were needed to have at least one daughter aged 9-15 years. Women with known cervical malignancy or acute medical condition were not eligible for the studies.

##### 4.1.1.1 *The Mendoza province*

The Mendoza province is located in the Cuyo region in the western-central part of Argentina with a total population of 1,885,000 inhabitants. According to national statistics, the mortality rate is slightly lower in Mendoza compared to overall figures from the country (7.3 versus 7.7 per 100,000). Maternal and infant mortality rates are also lower with 23 compared to 39 per 100,000 live births and 8.7 compared to 9.7 per 1,000 births, respectively. However, the illiteracy rate at 2.2% in Mendoza is slightly higher than the overall national rate of 1.9%. It has been estimated that 10.3% of the inhabitants in Mendoza do not have their basic needs met (173). A provincial program for cervical cancer prevention was established as early as 1991 in the Mendoza province (192), which was before the initiation of a national prevention program (170). The Mendoza is also one of the few provinces in the country with quality control of laboratories, as well as computer registration of cytology testing. Still, it has been reported that only 25% of all pap smears in Mendoza are performed on the target age-group (170).

#### 4.1.2 Study III

This was a descriptive population-based survey on 1510 women attending the cervical cancer screening program in five outpatient clinics located in Stockholm, Sweden. The outpatient clinics were located in the municipalities of Danderyd, Nacka, Sundbyberg, Stockholm City and Värmdö. Three clinics located in Jakobsberg, Botkyrka and Nynäshamn declined to take part in the study. The study invited women who were eligible for organized screening according to the national screening practices that were in use at the time of the study. Thus, women were required to be between 23 to 60 years of age and should not have had opportunistic screening recently.

#### 4.1.3 Study IV

This Swedish long-term prospective cohort study comprised 991 women with a diagnosis of CIN2 or CIN3 who had been treated with cervical conization surgery at Karolinska University Hospital Huddinge between 2000-2007 and Solna between 2006-2007. Eligible participants had to have i) undergone a cervical conization, ii) a histopathological diagnosis



of CIN2/3 in the punch biopsy before the conization or in the cone biopsy, and iii) have information on margin status of the cone biopsy in their medical record.

## **4.2 DATA COLLECTION**

### **4.2.1 Study I and II**

Using consecutive sampling, women were invited to participate from September to October 2011 (Study I) and February to April 2012 (Study II). Potential participants were approached in waiting rooms and consultations offices at the gynecological clinic and on Obstetrics/Gynecology wards. Women were informed about the purpose of the study and the anonymity of their responses and those who agreed gave oral informed consent. Data was collected through surveys and structured survey-based interviews conducted by the first author (Alder) in Study I and by surveys alone in Study II (supervised by Gustafsson). One author (Alder or Gustafsson) was always present during the completion of surveys to answer questions or explain parts of the survey that the respondents did not understand.

#### *4.2.1.1 Questionnaire*

To the best of my knowledge, there were no validated questionnaires regarding HPV vaccination acceptance when our studies were conducted. The questionnaires used in the studies were adapted from those used in two large population-based studies on HPV-vaccination on young adults and parents in Sweden (162, 163). In these studies, the questionnaires had been used for structured interviews as well as for web-based versions. They were developed at the Department of Medical Epidemiology and Biostatistics (MEB) Karolinska Institute in Sweden, and consent to use the questionnaires was given by one of its originators (Pär Sparén). The questionnaires were slightly modified to adapt it to the Argentinean population and thereafter translated to Spanish by one of the native Spanish-speaking authors (Perinetti). The revised questionnaires were pilot tested before use, which resulted in some simplifications of the language in Study I, while kept unchanged in Study II. The questionnaire used in Study I was divided into six sections: demographics (part 1), sexual behavior (part 2), awareness of HPV-associated disease (part 3), general perception of vaccination (part 4), acceptance of HPV vaccination (part 5) and screening practices (part 6). In Study II the questionnaire contained five sections: demographics (part 1), awareness of HPV-associated disease (part 2), general perception of vaccination (part 3), maternal acceptance of HPV vaccination for daughter (part 4) and perceptions on daughter's sexual activity (part 5). As in the Swedish studies, all women received information on the role of HPV as a causative agent of cervical cancer before they answered questions on HPV vaccination acceptance.

### **4.2.2 Study III**

The study was conducted between March 2013 and April 2014. Potential study participants were approached in waiting rooms of the outpatient clinics and those who agreed received surveys that they completed independently. Since one of the authors (Östensson or Alder) was present during the data-collection, it was possible to clarify misunderstandings and limit the number of missing responses. The average waiting and screening procedure time were measured on four separate occasions at two of the outpatient clinics on 197 women.

#### *4.2.2.1 Questionnaire*

A questionnaire for the purpose of this study was constructed by the authors, based on recommendations from the UK Working Party on patient-reported costs (193) and questionnaires previously used to assess knowledge and attitudes on HPV (194-196). A pilot trial was conducted on women with various occupations, which resulted in a subsequent refinement of the questionnaire through a validation process. The questionnaire was divided into three sections: demographics (part 1), time and travel costs and other direct non-medical costs of attending screening (part 2) screening compliance and knowledge of HPV and cervical cancer screening.

#### **4.2.3 Study IV**

Women eligible for the study were identified in the medical record based on International Classifications of Diseases (ICD) codes (N87.1; CIN2 or D06.9; CIN3) and intervention codes (LDC03; conization with diathermy or laser, or LDC00; conization with knife). Clinical data was collected from medical records from 1 November 2015 until 30 November 2017 and included: age, comorbidity, previous conizations, surgical modality, HPV status (when available), re-conizations and hysterectomy. Also, data from the pathology report was extracted on the diagnosis a) before treatment (based on histopathology or cytology), b) in the cone biopsy, c) during follow-up (cytology and histopathology), d) from re-conizations and hysterectomies, if any, and e) the margin status of the cone biopsy (positive or negative and endocervically or vaginally involved).

### **4.3 DEFINITIONS AND ASCERTAINMENTS OF EXPOSURE AND OUTCOME**

#### **4.3.1 Study I and II**

In both studies, acceptance of HPV vaccination (the outcome variable) was classified into three categories (unsure/unwilling to vaccinate, even if vaccination was free, willing only if vaccination was free and willing even if vaccination was not free).

#### **4.3.2 Study III**

Non-compliance with screening was defined as not attending screening within one year from the initial invitation. Knowledge of HPV and cervical cancer screening was assessed by the respondent's agreement with 17 correct facts. The answers were scored as 0 if the respondent did not agree or did not know, while agreement was scored as 1. Costs were expressed in 2012 prices and converted from Swedish kronor (SEK) to Euro (EUR) based on the average exchange rate for 2012 (1 EUR = 8.7 SEK). Travel cost for car users was estimated at EUR 0.45 per kilometer, on the basis of a formula developed by the Swedish Automobile Association, which included fuel, taxes, services and other costs (197). Travel cost for public transport was estimated at EUR 0.13 per kilometer, based on the cost of a 30-day public travel card (EUR 91.6), which is used by most travelers (70% of all ticket sales in Stockholm) (198) and mean distance to and from work by public transport (i.e. 24 kilometer) (199). Travel cost for taxi was based on the women's reported mean cost. Transportation for walkers and cyclist were valued at no costs. It was assumed that companions (if any) had the same travel cost as the screening participant (except when traveling together by car or taxi). Activities before and after screening were categorized into: work, education, leisure activity and dropping off or picking up children at school or child care. Costs related to arranged child care to facilitate screening attendance were estimated based on the women's reported mean

cost. The value of time off work for participants or their companion was estimated at EUR 29.9 per hour based on the Swedish gross wage rate (200). If it was not needed to take time off work, time was estimated at EUR 12.4 per hour, which is based on the value of non-working time used when assessing Swedish public transport projects (201, 202).

### **4.3.3 Study IV**

A negative margin was defined by the absence of high-grade dysplasia, CIN2 or worse, in the resection margin or by absence of CIN2 or worse in the endocervical curettage, performed after the conization. To verify that the primary exposure of interest – the margin status – was correctly assessed, all surgical pathology reports were re-viewed by one of the authors (Alder) and all uncertain cases were assessed by two authors (Alder and Andersson, the latter a senior gynecologist). Comorbidity was defined in terms of conditions likely to interact with the acquisition or persistence of HPV or development of CIN (i.e. autoimmune disorders, HIV, hepatitis B/C, malignancy, diabetes, genetic disorders and organ transplantation). HR-HPV positivity included also women infected with a probably carcinogenic HPV type, as many probably carcinogenic HPV types now have been reclassified as carcinogenic. The outcome was a histopathological diagnosis of residual or recurrent CIN2+ (CIN2, CIN3, AIS or worse, vaginal intraepithelial neoplasia (VAIN), vaginal cancer, or recurrence to the pelvis or other location). If both glandular and squamous components were reported, it was classified based on the glandular component (i.e. AIS and CIN was coded as AIS and ADC and SCC was coded as ADC). Information on residual or recurrent CIN2+ was obtained from the medical record and by linking the study women to the Swedish National Cervical Screening Registry (203), as described below.

## **4.4 OTHER METHODS**

### **4.4.1 Swedish National Cervical Screening Registry**

In Study IV, all women were linked to the Swedish National Cervical Screening Registry based on their unique registration numbers to obtain data on recurrent or residual disease (outcome). The Swedish National Cervical Screening Registry analyses and evaluates the national cervical cancer screening program. Their annual reports include information on screening coverage, invitations, laboratory reporting and other quality assessments. The registry has 100% coverage of all cervical cytology and histopathology tests in Sweden and thus provided complete data on these endpoints until Dec 31, 2016. Further, it has a nationwide coverage and provided information also on women who had migrated within the country, as data are linked to the unique registration numbers. As of 2015, the registry also includes data on all HPV tests performed throughout the country (187). Such information on the study women was also extracted from the registry, when available.

### **4.4.2 Conization procedures**

In Study IV, all women were treated by conization. The treatment modality in use at Karolinska Huddinge at the time of the surgeries was diathermy; mainly loop excision electrosurgical procedure using a Contoured loop excision of the transformation zone (C-LETZ) electrode (Utah Medical Products Inc., Midvale, UT, USA), while laser treatment was in use at Karolinska Solna (carbon dioxide laser (CO<sub>2</sub> laser)).

#### **4.4.3 HPV tests**

In Study IV, some women were HPV tested before treatment and during follow-up. The HPV tests in use at Karolinska at the time of the study were Hybrid Capture® 2 HPV DNA Test™ (QIAGEN Inc., Gaithersburg, MD, USA (previous Digene Corp.)), Linear Array® HPV Genotyping Test (Roche Molecular Systems Inc., Alameda, CA, USA) and Cobas 4800 (Roche Molecular Diagnostics, Pleasanton, CA, USA). Information on HR-HPV-status of the study women was also obtained from the Swedish National Cervical Screening Registry (203), as described above.

### **4.5 STATISTICAL ANALYSES**

In all studies, data frequencies of variables were calculated using SPSS (IBM, Armonk, NY, USA, IBM version 20.0 (Study I and II), IBM 21.1 (Study III) or IBM 25.0 (Study IV)). P-values were based on the Wald-Chi square or Fisher's exact test or Monte Carlo estimates when numbers were too small ( $n < 5$ ). All p-values were two-sided and  $< 0.05$  was considered statistically significant.

#### **4.5.1 Study I**

A multinomial cumulative logistic regression was used to explore potential correlates of HPV vaccination acceptance using SAS® (System 9.1; SAS Institute, Cary, NC, USA), with unsure/unwilling to vaccinate, even if vaccination was free, as the reference category. Exact estimates and p-values were obtained when frequencies were too low for multinomial regression. Statistically significant covariates from the multinomial regression (based on p-value  $< 0.05$ ) were included in the multivariable model, which adjusted for all other variables in the analysis.

#### **4.5.2 Study II**

Acceptance of HPV vaccination was classified into three categories, as described above. However, due to the fact that only a few women were unsure or unwilling to vaccinate their child, all women that answered such were excluded from the analysis on potential correlates of vaccination acceptance ( $n=18$ ). Therefore, a binomial logistic regression model was performed using SAS® (System 9.4; SAS Institute, Cary, NC, USA). Thus, this analysis was restricted to investigate correlates of willingness to pay for HPV vaccination, compared to willingness if vaccination was free. All statistically significant correlates from the binomial regression were included in the mutually-adjusted multivariable analysis.

#### **4.5.3 Study III**

A power calculation was performed before the study, which determined that a sample size of 1500 women would provide a power of 0.976 to detect a 10% difference in the proportion of correct answers on knowledge questions between age groups. Cronbach's alpha coefficient was used for internal reliability evaluation and an  $\alpha \geq 0.70$  was considered acceptable. Percentages were based on only those who responded (thus, missing data was not included).

Differences in mean transport costs by mode of travel, travel time and travel distance were analyzed by one-way analysis of variance (one-way ANOVA) at 5% level of significance, with a Bonferroni correction. Differences in sociodemographic characteristics of the study women were compared to the general female population in Sweden (based on official statistics from Eurostat (204)) using the Z-test. This was also used for comparing differences in HPV knowledge between age groups. In order to investigate correlates of HPV knowledge and screening compliance, a binomial logistic regression analysis was performed. Correlates of HPV knowledge was obtained by using a binary dependent variable based on the median knowledge score ( $\geq 5$  or  $< 5$ ). By using a manual backward stepwise procedure, non-significant variables were removed from the model or kept in the analysis if they changed the width of the other variables CIs by  $\geq 10\%$

#### **4.5.4 Study IV**

A Kaplan–Meier analysis was performed to plot curves of residual or recurrent CIN2+ among margin positive or negative women, respectively, and the log rank test was used to compare the distributions. The follow-up time was measured from the date of the first surgery until the date of CIN2+, death or deregistration or until end of study, Dec 31, 2016. Women with cervical cancer (SCC/ADC) in the cone biopsy were not included in the follow-up analysis. Cox proportional hazards models were used to assess HRs for the association between margin status and residual or recurrent CIN2+, adjusting for comorbidity, age at surgery, the severity of the lesion, treatment modality and previous conization. An additional Cox regression analysis was undertaken on margin status and recurrence/residual disease, stratified by HPV-status. Due to the limited sample, it was not possible to perform an HPV stratified analysis also on subdivided margin status.

### **4.6 ETHICAL CONSIDERATIONS**

#### **4.6.1 Study I and II**

Study I was approved by the Ethics Review Committee of the Diego Paroissien Public Hospital and Study II by the Ethics Review Committees of the Diego Paroissien Public Hospital and the Private Italian Hospital. Oral informed consent was obtained from all participants before administering the surveys/conducting the interviews.

#### **4.6.2 Study III**

Approval from the Regional Ethical Review Board of Stockholm was obtained, which determined that written informed consent was not required (Dnr: 04-679/3, 2010/944-32, 2012/1892-32). Both written and oral information about the purpose of the study was provided to all potential study participants and oral informed consent was obtained from all participants prior to the administration of the surveys.

#### **4.6.3 Study IV**

The study was approved by the Regional Ethical Review Board of Stockholm, which determined that informed consent from the participants was not required (Dnr: 168/03, 2004-679/3, 2010/944-32, 2013/763-32, 2014/2255-31/5 and 2017-2007/32). However, all women that had decided to block access to their medical record were excluded from the study.

## **5 RESULTS**

### **5.1 STUDY I**

#### **5.1.1 Characteristics of study sample**

In all, 228 women were invited to participate in the study, of which 200 (88%) agreed. Of these, 174 (87%) responded to the survey by interviews, while 26 (13%) responded by written surveys. Due to a high frequency of missing data of the written surveys, only women administered by interview were included in the study. Thus, the final study sample consisted of 174 women. The median age of the women was 23 years. Most women were married or in a relationship (84%) and 71% were housewives/unemployed. Half the sample (50%) had an annual income of <170 EUR and almost two-thirds (61%) reviewed welfare and had less than a high school education (65%).

#### **5.1.2 Acceptance of HPV vaccination**

Acceptance of HPV vaccination was 95% if vaccination was free and 75% stated that they were also willing to pay for vaccination. Few (5%) were unsure/unwilling to be vaccinated against HPV. Among participants willing to pay for vaccination, the median price women were willing to pay for vaccination was 11.50 EUR (per dose).

#### **5.1.3 Correlates of HPV vaccination acceptance**

General believes in vaccination safety and vaccine effectiveness was statistically significantly positively associated with acceptance of HPV vaccination, irrespective of costs in the univariate analysis. Also based on results from the univariate analysis, negative associations were found between receiving welfare as well as not having heard of condyloma and willingness to pay for vaccination, however, CIs were wide. In the multivariable analysis, after adjustment for all other variables in the analysis, general believes in vaccination safety remained statistically significant. Women who did not regard vaccination as safe were statistically significantly less willing to accept vaccination if it was free (OR: 0.07, 95% CI: 0.01-0.49), and if it was not free (OR: 0.15, 95% CI: 0.03-0.88). Also being a welfare recipient remained statistically significantly negatively associated with vaccination acceptance if vaccination was not free (OR: 0.11, 95% CI: 0.11-0.99) in the multivariable analysis (Table 2).

Table 2. Multivariable analysis on acceptance of human papillomavirus (HPV) vaccination with 'unsure/unwilling even if vaccination was free' as reference category.<sup>a</sup>

	Willing only if vaccination was free		Willing even if vaccination was not free		p-value
	OR	95% CI	OR	95% CI	
Believes vaccination to be a safe method to prevent disease					
Very or fairly safe	<b>1</b>		<b>1</b>		0.01
Not so safe or not safe	<b>0.07</b>	<b>0.01-0.49</b>	<b>0.15</b>	<b>0.03-0.88</b>	
Don't know	0.23	0.03-2.07	<b>0.05</b>	<b>0.01-0.51</b>	
Welfare recipient					
No	1		<b>1</b>		0.00
Yes	0.53	0.05-5.38	<b>0.11</b>	<b>0.01-0.99</b>	
Heard of condyloma (genital warts) prior to study <sup>b</sup>					
Yes	1		1		0.0
No	2.18	0.00-29.52	0.27	0.00-1.94	

<sup>a</sup>All odds ratios are mutually adjusted for all other variables in the table. <sup>b</sup>Analyses conducted using exact estimates. OR, odds ratio; CI, confidence interval. Statistically significant OR and CI are marked in bold.

#### 5.1.4 Perceptions of HPV vaccination

Nearly half the women (44%) were under the impression that HPV vaccination would provide complete protection against cervical cancer. The majority (74%) stated that they would not practice more unsafe sex following HPV vaccination, while one in ten (10%) believed they would. The main reasons for abstaining from HPV vaccination were concerns regarding side-effects (54%) and concern about the protective effect (16%).

#### 5.1.5 Knowledge of HPV and cervical cancer and screening practices

Almost all women (95%) were aware of cervical cancer screening prior to the study, eight out of ten (81%) were aware of cervical cancer, 58% were aware of HPV and 24% were aware of condyloma. More than half (55%) had ever participated in screening and participation rate was found to increase with age; 29% in women aged 18-19 years and 84% in women aged 26-30 years. Only 4% of the non-participants in screening stated that they would not participate at all in the future if they were vaccinated against HPV. However, 24% of the previously screened women believed that they would participate less frequently.



## **5.2 STUDY II**

### **5.2.1 Characteristics of study sample**

Of the 211 mothers invited to the study, 20 (10%) did not agree to participate and another 11 (5%) were excluded due to a high frequency of missing data. The final sample thus comprised 180 women, of which the majority were recruited from the public hospital (86%) and 14% from the private hospital. The median age of the women was 37 years (range 26-59 years). Most mothers were married or in a relationship (79%), more than half were housewives (57%) and nearly half had four or more children (45%). Only one third (27%) had high school education or above.

### **5.2.2 Maternal HPV vaccine acceptance**

Nine out of ten (90%) mothers stated that they were willing to vaccinate their child against HPV. Sixty percent stated that they would do so even if vaccination were not free. Only three mothers were unwilling to vaccinate even if vaccination was free. The mean price that mothers were willing to pay for vaccination was 23 EUR (per dose). Still, 21 mothers stated that they would vaccinate their daughters irrespective of costs.

### **5.2.3 Correlates of willingness to pay for vaccination**

Higher education, being gainfully employed, having a higher disposable household income and being aware of cervical cancer prior to study were all statistically significantly associated with willingness to pay for HPV vaccination in the univariate regression analysis. Also, there were tendencies of higher vaccination acceptance even if vaccinating was not free, among older women and women with previous HPV awareness, although it did not reach formal statistical significance. Similarly, mothers who were unsure about vaccine safety in general seemed less willing to pay for vaccination but this was neither statistically significant. All variables that did reach statistical significance were included in the mutually adjusted multivariable model. In this analysis, being gainfully employed (OR: 2.54, 95% CI: 1.01-6.38), having a higher disposable household income (OR: 3.28, 95% CI: 1.36-7.94) and being aware of cervical cancer prior to study (OR: 3.22, 95% CI: 1.02-10.14) remained statistically significantly associated with willingness to pay for vaccination.

### **5.2.4 Perceptions of HPV vaccination**

Almost half (46%) believed their daughter to be fully protected against cervical cancer after vaccination, while few (10%) believed that they would not. Half of the women (54%) considered vaccination to be safe in general while 12% believed it to be not so safe/unsafe. Further, 65% considered vaccines in general to be effective, while few (5%) regarded vaccination as not so effective/ineffective. The concerns related to HPV vaccination included vaccine efficacy (41%), side-effects (27%) and whether additional doses were needed (17%). A large proportion (39%) of the mothers were concerned as to whether their daughters would be more likely to engage in sexual activity following HPV vaccination. Still, most women (64%) stated they would not let this concern make them abstain from HPV vaccinating their daughter.

### **5.2.5 Awareness of HPV and its related diseases**

The majority of the mothers had previous awareness of cervical cancer (83%), just over half were aware of HPV (56%) and 39% were aware of condyloma before the onset of the study. Among those aware of HPV, 64% thought it could cause cervical cancer.

## **5.3 STUDY III**

### **5.3.1 Characteristics of study sample**

Among the invited women, around 2% declined to participate and the final study sample comprised 1510 women. The study women had a statistically significant higher level of education and income in comparison to the general female population in Sweden, the Stockholm County and all municipalities included in the study except Danderyd ( $p < 0.01$ ).

### **5.3.2 Travel characteristics, time and travel costs and other direct non-medical costs**

One third of the women went to and from screening using public transport (30%), another third (28%) went by car and yet another third (27%) walked. Among those who went by public transport, most women ( $n=628$ ) paid with a 30-day pass and few ( $n=40$ ) paid with a zone ticket (cost range: EUR 4-6). The majority came directly from their homes (86%) and 12% came from work. After the clinic visit, 60% of the women stated that they were traveling to work, while 28% were going back to their home. Mean distance traveled to and from the clinic was 6.4 and 11.4 km, respectively, with a mean total distance of 17.8 km. Average travel times to and from the clinic were 18 and 26 minutes (not including time related to errands on the way), resulting in a total travel time of 44 minutes. When comparing mean travel time, travel distance and travel costs by mode of transport, all differences were statistically significant.

The estimated average waiting time was 10 minutes and the estimated time of screening procedure was 13 minutes, resulting in a total time of 23 minutes/visit. Just over half (53%) the women took time off work to attend screening, with a mean time of 147 minutes. Twelve percent had a companion with them to screening (most were accompanied by their partner). A large share of the companions (59%) had also taken time off work, with a mean time of 113 minutes. Relatively few women (3%) had arranged child care to facilitate screening attendance. Of those 42 who did, 35 incurred out-of-pocket costs related to this (mean time: 168 minutes, estimated cost: EUR 78.4). Mean total travel cost to the screening clinic was estimated at EUR 1.9 and 2.9 from the clinic. Estimated mean total cost (time and travel costs and other direct non-medical costs) per screening attendance was EUR 50.8. If also including companions (if any) this amounted to EUR 55.6 per attendance.

### **5.3.3 Knowledge of cervical cancer screening and HPV**

Many women (69%) knew that the purpose of screening was to prevent cervical cancer while one third (31%) were under the impression that its purpose was to prevent all types of gynecological cancers. HPV knowledge was compared between age groups, and a statistically significantly higher level of knowledge was found in younger women aged 29 years or below compared to women aged 30 to 49 years in regards to HPV being transmitted sexually (51% versus 39%), HPV infecting both sexes (30% versus 24%) and HPV being most common among young (37% versus 27%). This was also found for knowledge on HPV being mostly asymptomatic (41% versus 35%), that persistent infection with HPV can lead to dysplasia (54% versus 49%) and that HPV can cause condyloma (24% vs 22%). Among all women, 64% had knowledge on the existence of HPV vaccines; but less (41%) knew that it is most effective if administered prior to sexual debut and that HPV vaccination does not provide complete protection against all HPV types (34%). Only 7% of women regarded their

knowledge of HPV and cervical cancer as good, while the corresponding figure was slightly higher for knowledge of cervical cancer prevention (16%). Many of the respondents (63%) wanted to obtain additional information on HPV from a midwife or a physician and 52% wanted to receive information through brochures.

#### **5.3.4 Screening compliance**

In all, 44% of the women stated that they were non-compliant to screening. Among those, just over half (51%) expressed that this was due to difficulties in taking time off work, another 33% were “too busy” and 16% stated other reasons (e.g. fear of gynecological examination, previous negative experiences, menstrual period and pregnancy).

#### **5.3.5 Correlates of screening compliance and HPV knowledge**

There was a statistically negative association between screening compliance and need to take time off work, and screening compliance was found to decrease with increasing number of hours needed (Table 3). The OR was 0.62 (95%CI: 0.43-0.89) for taking one hour off work, 0.46 (95% CI: 0.34-0.62) for two hours off work and 0.27 (0.18-0.39) for three hours off work compared to women not taking time off work. Taking four hours or more off work was also statistically significantly associated with lower screening compliance compared to not taking time off work (OR 0.40, 95% CI: 0.27-0.60), although the OR was not lower than OR for women taking three hours off work. Bringing a companion to screening was also statistically significantly negatively associated with screening compliance (OR: 0.51, 95% CI: 0.36–0.71), while HPV knowledge was positively associated (OR 2.4, 95% CI: 1.91–3.02).

Higher education was statistically significantly associated with HPV knowledge and increased with higher level of education; OR for women with high school or equal was 3.23 (95% CI: 1.56–6.66) and 8.67 (95% CI: 4.19–17.93) for women with more than high-school education compared to women with less than high school education. Lower age was also statistically significantly associated with screening compliance, with OR of 1.9 (95% CI: 1.01-3.55) in women aged 24 years or below and 1.89 (95% CI: 1.09–3.28) in ages 25-29 years, compared to women aged 55 years or above. Knowledge was also higher in women aged 45-54, compared to women aged 55 years or above, while no statistically significant differences could be shown in women aged 30-44 years.

Table 3. Correlates of human papillomavirus (HPV) knowledge and screening compliance for 1510 women attending the clinic-based cervical cancer screening program in Sweden.

Parameters	HPV knowledge <sup>a</sup> OR (95% CI)	Screening compliance <sup>a</sup> OR (95% CI)
<b>Age (years)</b>		
≥55	Reference	Reference
≤24	1.9 (1.01–3.55)	.43 (.23–.82)
25–29	1.89 (1.09–3.28)	.89 (.51–1.53)
30–34	1.52 (.91–2.55)	1.37 (.83–2.27)
35–39	1.32 (.78–2.23)	1.1 (.66–1.86)
40–44	1.39 (.83–2.35)	1.66 (.99–2.77)
45–49	1.98 (1.17–3.37)	2.1 (1.24–3.53)
50–54	2.35 (1.28–4.30)	1.23(.68–2.22)
<b>Education level</b>		
<High school	Reference	Reference
High school or equal	3.23(1.56–6.66)	1.72(.92–3.19)
>High school	8.67 (4.19–17.93)	1.32(.70–2.46)
<b>Total annual income (€)</b>		
<13 783	Reference	Reference
13784–27 568	1.03 (.64–1.65)	0.8 (.49–1.28)
27569–41 353	.66 (.43–1.02)	1.76 (1.13–2.75)
41 354–55 137	.54 (.34–.87)	1.45 (.90–2.33)
55138–68 922	.57 (.34–.94)	1.62 (.95–2.74)
68923–82 707	.28 (.15–.53)	1.07 (.56–2.05)
82708–96 492	1.54 (.66–3.62)	1.26 (.56–2.84)
96493–110 276	.23 (.07–.74)	2.43 (.74–8.02)
≥110277	.38 (.14–1.03)	1.32 (.46–3.77)
<b>No time off work</b>		
1 hour off work		Reference
2 hours off work		.62 (0.43–.89)
3 hours off work		.46 (0.34–.62)
>4 hours off work		.27 (.18–.39)
<b>No companion</b>		
Companion		Reference
No HPV knowledge*		.51 (.36–0.71)
HPV knowledge		Reference
		2.4(1.91–3.02)

OR = odds ratio. CI = confidence interval.

<sup>a</sup>Two separate models were generated for the cohort after manual backward stepwise selection excluding non-significant variables. Therefore, not all variables were applicable in both models.

\*Correlates of knowledge were determined using a dichotomous dependent variable based on the median (i.e., ≥ 5 or <5) HPV knowledge score. Therefore, "knowledge of HPV" is referred to ≥5 on the HPV knowledge score and "No knowledge of HPV" referred to <5 on the HPV knowledge score.

## **5.4 STUDY IV**

### **5.4.1 Characteristics of study sample**

Of the 1075 study women, 991 met the inclusion criteria of which 721 women had undergone treatment at Karolinska Huddinge and 270 at Karolinska Solna. At the end of the study, 4% were deceased and 2% were deregistered, mainly due to migration. The median age was 33 years (range 19-94 years). The majority of women had no underlying comorbidity (91%). Few (3%) had previously been treated with conization. The most common reason for conization was CIN3 (62%), followed by CIN2 (30%) and CIN1 (5%). The majority was treated by diathermy (73%) and 27% by laser. Hospital site, age at surgery, treatment modality, previous conization treatment and the reason for conization treatment were all statistically significantly associated with margin status.

### **5.4.2 Margin Status of the cone biopsy**

In all, 65% of cone biopsies had negative margins, while 31% were incomplete at one or more margins, or of uncertain margin status, and additionally 4% were not eligible for margin status evaluation, due to absence of dysplasia in the cone biopsy. Of the samples with positive or uncertain margin status, 13% affected the endocervical margin, 7% the vaginal margin, 6% both margins and in another 4% it was unclear from the report which margin that was affected. The histopathological diagnosis of the cone biopsies were CIN3 in 63%, CIN2 in 22%, CIN1 in 7% and SCC/ADC in 3% of cases. There was a statistically significant association with the severity of lesion and margin involvement ( $p<0.001$ ).

### **5.4.3 Re-conization, hysterectomy and follow-up HPV**

One in ten women (11%) underwent re-conization treatments. There was a larger share of women with involved margins in the re-treatment group compared to those without re-treatment (51% and 28%,  $p<0.001$ ). One third underwent re-conization due to CIN3 (30%), another third due to CIN2 (27%) and one forth due to CIN1 (25%). Half the re-conization biopsies showed only CIN1 or no dysplasia (51%), while 46% showed CIN2-3. Only every fifth women (20%) were HPV tested at any time during follow-up and 9% were HR-HPV positive. One in ten women (9%) underwent a hysterectomy during follow-up. Reasons for hysterectomy were residual or recurrent dysplasia (33%), treatment for SCC/ADC (22%) and other reasons (24%). Notably, another 21% underwent hysterectomy only on the basis of involved margins in the previous cone biopsy.

### **5.4.4 Follow-up and outcome**

Median follow-up time was 10 years (range 0-16 years). Most women (88%) were followed 6 to 16 years. In total, 12% of the women were diagnosed with a histologically confirmed residual or recurrent CIN2+ during follow-up (all 25 women with SCC or ADC in the first conization were excluded from this analysis). Nearly half (46%) of those who relapsed did so within the first year, while 16% relapsed at least 5 years later. There was a higher proportion of involved margins in the recurrence group compared to the non-recurrence group (51% versus 26%,  $p<0.001$ ). Only 1% was diagnosed with cervical cancer during follow-up. There was a tendency toward a higher proportion of involved margins in the cervical cancer group; however, it was not statistically significant ( $p=0.074$ ).

#### 5.4.4.1 Kaplan-Meier analysis

The Kaplan-Meier curve of the association between margin status and time to residual or recurrent CIN2+ is illustrated in Figure 7A, and according to subcategorized levels of margin status in Figure 7B. A statistically significant difference was observed according to margin status with the log rank test in both analyses ( $p < 0.001$ ).

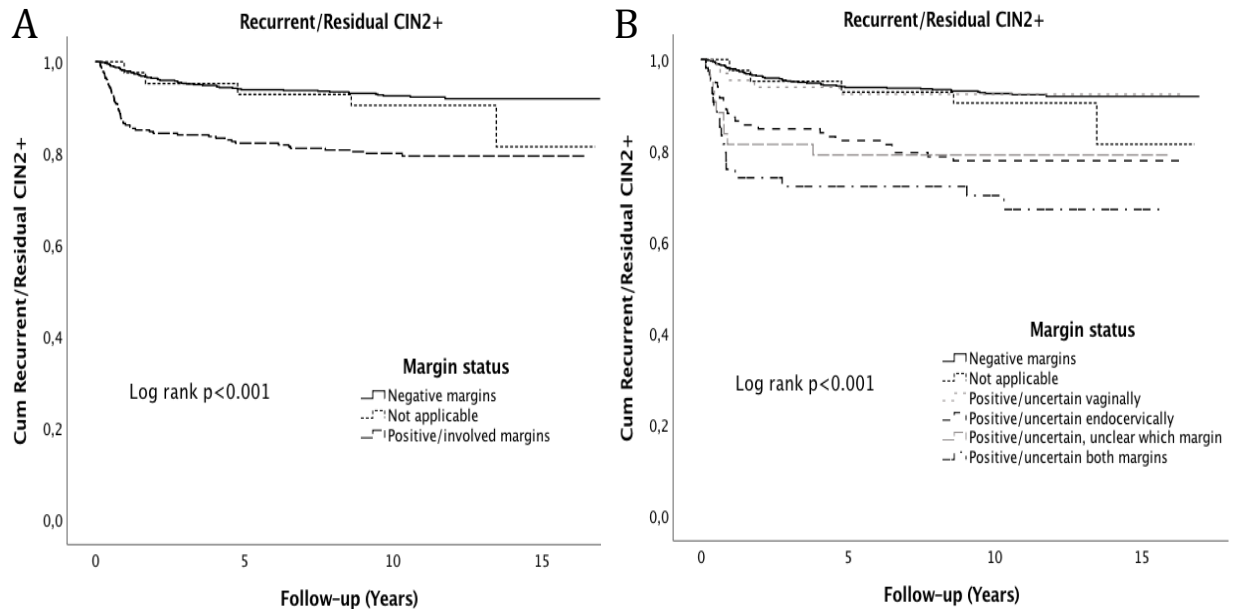


Figure 7.A. Kaplan-Meier plot of residual or recurrent cervical intraepithelial neoplasia or worse (CIN2+) according to margin status. B. Kaplan-Meier plot of residual or recurrent CIN2+ according to different levels of margin status.

#### 5.4.4.2 Cox proportional hazards models

Involved margins were positively associated with residual or recurrent CIN2+ (HR 2.68, 95% CI: 1.82-3.94  $p < 0.001$ ) in the multivariate Cox model (adjusting for comorbidity, age at surgery, the severity of the lesion, treatment modality and previous conization procedures), (Table 4). Other covariates associated with risk of CIN2+ were comorbidity (HR 2.29, 95% CI: 1.39-3.75), and previous conization procedure/-s (HR 2.28, 95% CI: 1.07-4.85). The subcategorized levels of margin status also remained statistically significant in the adjusted Cox regression analysis ( $p < 0.001$ , Table 5). An almost three-fold risk of residual or recurrent CIN2+ was shown among women with involved endocervical margins (HR 2.74, 95% CI: 1.69-4.45) and in women with involved margins though uncertain if endocervical or vaginal (HR 2.84, 95% CI: 1.39-5.80) and even higher risks when both margins were involved (HR 4.97, 95% CI: 2.84-8.69) compared to women with negative margins. No statistically significant positive association with residual or recurrent CIN2+ was found when only the vaginal margin was involved. Also in this model, comorbidity and previous conization procedure/-s were statistically significant covariates.

Table 4. Cox regression model for Hazard Ratio (HRs) of residual or recurrent cervical intraepithelial neoplasia or worse (CIN2+)<sup>a</sup>.

	Adjusted HR*	95% CI	P-value
<b>Margin status</b>			<0.001
Negative	1		
Not applicable	1.95	0.51-7.45	
Positive/uncertain	<b>2.68</b>	<b>1.82-3.94</b>	
<b>Comorbidity</b>			0.001
No comorbidity	1		
Comorbidity	<b>2.30</b>	<b>1.40-3.77</b>	
<b>Age at surgery</b>			0.830
19-39	1		
40+	1.05	0.69-1.60	
<b>Diagnosis in cone biopsy</b>			0.262
CIN1/no dysplasia	1		
CIN2	1.27	0.43-3.76	
CIN3+	1.78	0.65-4.89	
<b>Mode of surgery</b>			0.669
Diathermy	1		
Laser	1.21	0.80-1.82	
Ultrasounds knife	0.00	0.00-4.50E+161	
<b>Previous conization</b>			0.032
No	1		
Yes	<b>2.28</b>	<b>1.07-4.85</b>	

<sup>a</sup>All women diagnosed with SCC/ADC in the original conization surgery were excluded (n=25);

\*Hazard ratios are adjusted for all variables in the analysis; CI, confidence interval. Statistically significant HR, and CI and are marked in bold.



Table 5. Cox regression model for Hazard Ratio (HRs) of residual or recurrent cervical intraepithelial neoplasia or worse (CIN2+) according to subcategorized levels of margin status <sup>a</sup>.

	Adjusted HR*	95% CI	P-value
<b>Margin status</b>			<0.001
Negative	1		
Not applicable	1.93	0.50-7.37	
Positive/uncertain vaginally	0.96	0.38-2.42	
Positive/uncertain, uncertain which	<b>2.84</b>	<b>1.39-5.80</b>	
Positive/uncertain <u>endocervically</u>	<b>2.74</b>	<b>1.69-4.45</b>	
Positive/uncertain both	<b>4.97</b>	<b>2.84-8.69</b>	
<b>Comorbidity</b>			0.001
No comorbidity	1		
Comorbidity	<b>2.29</b>	<b>1.39-3.75</b>	
<b>Age at surgery</b>			0.885
19-39	1		
40+	0.97	0.64-1.48	
<b>Diagnosis in cone biopsy</b>			0.279
CIN1/no dysplasia	1		
CIN2	1.24	0.42-3.65	
CIN3+	1.73	0.63-4.75	
<b>Mode of surgery</b>			0.595
Diathermy	1		
Laser	1.24	0.82-1.88	
Ultrasounds knife	0.00	0.00-1.78E+187	
<b>Previous <u>conization</u></b>			0.047
No	1		
Yes	<b>2.14</b>	<b>1.01-4.55</b>	

<sup>a</sup>All 25 women diagnosed with SCC/ADC in the conization surgery were excluded (n=25); \*Hazard ratios are adjusted for all variables in the analysis; CI, confidence interval. Statistically significant HR, and CI and are marked in bold.

An additional Cox regression model, stratified by HPV-status, was performed to further analyze the association between margin status and recurrent/residual disease (Table 6). Margin status remained statistically significant when no follow-up HPV test was taken ( $p<0.001$ ). There was also a tendency towards increased risk among margin positive women who were HR-HPV positive during follow-up, although it did not reach statistical significance (2.21, 95% CI: 0.98-5.01,  $p=0.031$ ).

Table 6. Cox regression model for Hazard Ratio (HRs) of residual or recurrent cervical intraepithelial neoplasia or worse (CIN2+) stratified by HPV status<sup>a</sup>.

	No <u>HR-HPV</u> test (n=777)		<u>HR-HPV</u> positive (n=84)		<u>HR-HPV</u> negative (n=105)	
	Adjusted HR* (95% CI)	P-value	Adjusted HR* (95% CI)	P-value	Adjusted HR* (95% CI)	P-value
<b>Margin status</b>		<0.001		0.031		0.455
Negative	1		1		1	
Not applicable	0.58 (0.05-6.60)		<b>16.02 (1.35-190.13)</b>		0.748 (0.04-13.32)	
Positive/uncertain	<b>3.32 (1.99-5.51)</b>		2.21 (0.98-5.01)		2.021 (0.66-6.15)	

<sup>a</sup>All women diagnosed with SCC/ADC in the original conization surgery were excluded (n=25); \*Hazard ratios are adjusted for age at surgery, diagnosis in cone biopsy, mode of surgery and previous conization; CI, confidence interval; HR-HPV, High-risk Human papillomavirus. Statistically significant HR, and CI and are marked in bold.

## 6 DISCUSSION

### 6.1 STUDY I

#### 6.1.1 Main findings and interpretations

We found that acceptance of HPV vaccination was very high in this Argentinean setting; with 95% of young adult women stating that they would accept vaccination. The majority (75%) expressed that they were also willing to pay for vaccination. The latter is an especially important finding since there is no subsidized catch-up vaccination among young adult women in Argentina. However, the price these women stated that they were willing to pay for vaccination was four times lower than the actual price (205). Also, we found that women who received welfare were less willing to pay for vaccination, although the CI was wide but still reached a significant level. Taken together, these findings could implicate that cost could pose a barrier to vaccination of young adult women in this setting.

Another important finding was that acceptance of HPV vaccination was lower among women who did not regard vaccines as safe in general. Also, half the respondents stated that the main reason to abstain from vaccination was concerns regarding side-effects. These findings suggest that it is important to ensure the safety of HPV vaccines in order to achieve a high vaccination uptake. A common misconception was that HPV vaccination would provide complete protection against cervical cancer, which it does not. This needs to be clarified to potential vaccine receivers. Also, one in ten women stated that they would practice more unsafe sex if they were vaccinated, which also needs to be targeted. It is therefore important to develop public education campaigns.

It was very encouraging that almost all women were aware of cervical cancer screening and eight out of ten women were also aware of cervical cancer. It should be noted that the commonly used name for cervical cancer screening in Argentina is *Papanicolaou*, while the word for cervical cancer is *cancer de cuello uterino*, which could explain this discrepancy. Overall awareness of HPV was also surprisingly high (58%) among our study women. However, this high figure relates only to awareness, i.e. having heard of HPV.

#### 6.1.2 Comparisons with other studies

Overall acceptance of HPV vaccination was higher in our study compared to findings from the only existing study on HPV vaccination of adult women from Argentina (95% versus 75%, respectively). That study was conducted in the Buenos Aires area in 2012, and investigated 1,200 mothers' willingness to vaccinate themselves or daughters (185). Our higher findings on acceptance could be influenced by the fact that all women received information on the role of HPV as a causative agent of cervical cancer before they answered questions on HPV vaccination acceptance. This could demonstrate the importance of providing adequate information about the vaccine. Further, our study included only women aged 30 years or below while the other study included women up to age 49 years. Several studies have observed a negative association between higher age and acceptance (161, 163). Also, the women in our study had a lower level of education, which has been reported to be associated with higher vaccination acceptance among young adults (163).

As many as three out of four women in our study were willing to pay for vaccination, which is higher than corresponding figures from Sweden, despite our study women's limited economic means. In the Swedish study, only 34% of young adult women were willing to pay

for vaccination (163). Reasons for this might be differences in attitudes regarding government expenditure as well as differences in cervical cancer burden between the countries. Still, the importance of cost in relation to high vaccination acceptance has been shown in several other studies (160, 161, 163). Also, in the Buenos Aires study (185) cost was found to constitute a barrier to HPV vaccination and was the second most common reason for non-vaccination. The finding that acceptance of HPV vaccination was lower among women who do not regard vaccines as safe in general has also been shown in several other studies (160, 162, 163). The misconceptions of HPV vaccination were higher in our study compared to findings in other studies. Almost half of our women believed HPV vaccination to provide complete protection against cervical cancer, which is considerably higher compared to European findings of 7-8% (163, 206). Cervical cancer screening awareness was equally high in a previous Argentinean study from 2003, in which 93% were aware of it (207). In a recent Argentinian study on knowledge of HPV on almost 1,300 respondents, a majority (62%) had knowledge of the relation between HPV and cervical cancer (208). This is very promising and could suggest an increased knowledge among the population. In our study less (58%) were aware of HPV, i.e. knew that it existed, while having knowledge of HPV being a causative agent of cancer is a higher level of knowledge.

### **6.1.3 Methodological considerations and validity**

A major strength of this study is that it is one of few studies on HPV vaccination acceptance from Argentina. Also, it is the only study from a non-metropolitan part of the country, which is important as cervical cancer burden varies widely throughout the country, and Buenos Aires is one of the province with the lowest cervical cancer burden (169). Another strength is that all included women in this study were administered by the structured interview-technique, which resulted in almost no missing data. Furthermore, the non-participation rate was good (76%), even though we excluded all written surveys.

An important methodological aspect one has to consider is the validity of the survey. At the time of the study, there were no validated questionnaires on HPV vaccination acceptance. However, the survey used in this study was adapted from a questionnaire used in a large Swedish population-based study (17), as described more in detail in the methods section. It was developed at MEB together with their questionnaire advisory group and used on more than 10,000 respondents in the Swedish study. We also performed a pilot trial of our translated and slightly modified version that did not reveal any obvious misconceptions. Overall, it was a well-developed, comprehensive survey, used on a large number of women prior to our study, which we consider to be a strength in our study. It was noted in our study, that a recurring spontaneous concern was high cost, which was not listed as a multiple-choice option. It is likely that more women would have stated this as a concern if it had been included as a multiple-choice option. Also, the predictive validity of our findings on vaccination acceptance can be discussed. This study examined theoretical acceptance, which may not be transferable to actual uptake. This could be especially important for willingness to pay for vaccination, especially since the actual price was not specified. Further, the results could have been influenced by the fact that we explained the causative role of HPV in cervical cancer development. Also, women might have considered acceptance to be the socially desirable response, which could have influenced our results. The latter could be especially important as the surveys were administered by interview. Nowadays there are several validated scales for HPV vaccine surveys (209, 210), to reduce methodological issues related to the design of questionnaires.

There are also several general limitations to this study. The cross-sectional design, small

sample size and the homogeneity of the study population limit the generalizability of our findings. Also, there are several potential sources of selection bias including the limited geographical area represented by the inclusion of women from only one hospital and that all women had actively sought health care.

## **6.2 STUDY II**

### **6.2.1 Main findings and interpretations**

Maternal acceptance of HPV vaccination was very high both when free of charge (90%) and when it was not free (60%). The high level of acceptance is of major importance for a country like Argentina with a high burden of cervical cancer and insufficient cervical cancer screening coverage. We found that women who were gainfully employed and had a higher disposable household income were more willing to pay for vaccination, as were women with prior awareness of cervical cancer. The finding of an association between employment status and income with willingness to pay for vaccination suggests that cost could be an obstacle to catch-up vaccination. There is no subsidized catch-up vaccination for girls over the age of 11 years in Argentina, and vaccination is to be self-financed by the families.

Also, women with prior awareness of cervical cancer were more willing to pay for HPV vaccination, indicating the importance of improving awareness of HPV and its related diseases. Our findings showed that four out of five mothers were aware of cervical cancer prior to the study, which is equally high as in Study I. Also, awareness of HPV was equally and surprisingly high as in Study I, with more than half (56%) having heard of it. Among those aware of HPV, a very high figure (64%) believed it could cause cervical cancer.

A very discouraging finding was that only half the mothers believed vaccination to be safe in general and only a slightly higher number found vaccination to be effective in general. Also, 41% expressed concerns about vaccine efficacy and almost one third regarding side-effects. In our study, there was a tendency towards lower acceptance of vaccination among mothers with general disbelieves in vaccination safety; however, it did not reach formal statistical significance. Still, belief in vaccination safety was found to be correlated with acceptance in our previous study, both when vaccination was free of charge and when it was not. Several other studies have also found beliefs in vaccination safety to be an important factor of HPV vaccination acceptance (160, 162, 163). It is thus important to target these safety issues in order to achieve high vaccination uptake.

Almost half the mothers incorrectly believed their daughter to be fully protected against cervical cancer after vaccination, while few believed that they would not. Also, a large proportion of the mothers were concerned as to whether their daughters would be more likely to engage in sexual activity following HPV vaccination. Still, most women stated they would not let this concern make them abstain from HPV vaccination.

### **6.2.2 Comparisons with other studies**

Many studies from low-income settings have shown equally high figures on parental vaccination acceptance (109, 211-214). On the contrary, a lower maternal acceptance rate (75%) was found in the study from Buenos Aires (185), as discussed in Study I. A more recent Argentinean study on HPV vaccination acceptance from the Chaco province found an even lower parental acceptance rate of just below 50% (215). Studies on the actual uptake of HPV vaccination after the implementation of HPV vaccine to the organized school vaccination program are scarce. It has been reported that full-course vaccination coverage was only 50% in the 2000 birth cohort (118), and even lower local figures have been presented, with an uptake of only 25% in the Chaco province based on data from 2012 (215).

A concern in connection with the launching of vaccines was that vaccinated girls might engage in more sexual risk-taking. Among our study women, this was a quite common

concern. Several studies, among them a large study from Norway (216), have shown that the subsequent sexual behavior among HPV vaccinated individuals is unchanged, and no different compared to unvaccinated.

### **6.2.3 Methodological considerations and validity**

The strength and limitations in terms of the study design and questionnaire are at large similar to what's described for Study I. In contrast to Study I, all data was collected through written questionnaires instead of interviews. This resulted in more missing data but even after excluding questionnaires with too much missing data, the participation rate was still at high 85%. On the other hand, the use of questionnaires might reduce reporting bias and social desirability bias. The women might have perceived that acceptance was the socially preferred answer, and might have felt pressured to answer "correctly", and this source of error could have been lower for the written questionnaire technique. This might also be the case for potential bias related to questions regarding sensitive matters such as income or daughters sexual history. Acceptance was slightly lower in this study compared to Study I, yet the use of questionnaire or interview-based technique have probably not influenced these results in any major way.

Another important aspect was that there was an uneven distribution of study participants, with 86% from the public and the 14% from the private sector. However, we believe that this may be fairly representative, as women of low socioeconomic status constitute a large part of the Argentinean population.

A major limitation of this study was that the regression analysis was limited to investigate only correlates of willingness to pay for HPV vaccination, compared to willingness if vaccination was free. It would have been interesting to investigate correlates of vaccination for free compared to those who were unsure or unwilling to vaccinate their daughter, especially since vaccination is free of charge in the school-based vaccination program. However, due to few numbers, we were not able to perform this analysis.

## **6.3 STUDY III**

### **6.3.1 Main findings and interpretations**

This study found that the estimated mean total time and travel costs and direct non-medical cost for women attending cervical cancer screening in Sweden was substantial and might be an obstacle to screening and may affect the overall cost-effectiveness.

Another important finding was that nearly half the women did not attend screening within one year from their invitation, of which half cited difficulties in taking time off work. Also, there was a statistically negative association between screening compliance and need to take time off work, and screening compliance decreased with increasing number of hours needed off work. This suggests that difficulties in taking time off work could constitute a barrier to screening. Increased flexibility by extended opening hours is one possibility to target this issue.

Also having a companion to screening correlated negatively with screening participation. In our study, one in ten women brought a companion and it is important to understand the reason for this. Among women who did not attend screening within one year from their invitation, 16% expressed other reasons, including fear of gynecological examination and previous negative experiences. The choice of having a companion might be due to such reasons. This is something that should be further investigated.

Another finding of importance was that women with higher HPV knowledge had higher screening compliance. HPV knowledge, in turn, was higher among younger women and women with higher level of education. Still, overall knowledge of HPV was quite low. Less than half knew that HPV is transmitted sexually, that it can infect both men and women and that it could cause dysplasia. Also, one third of the women did not know the purpose of screening, but believed it to protect from all gynecological cancer. Over 60% were however aware of the existence of HPV vaccines. Education is thus needed and may be used to improve screening participation.

### **6.3.2 Comparisons with other studies**

The importance of costs as a barrier to screening was shown when a fee was introduced for cervical cancer attendance in Stockholm. The participation rate fell by 23% but rose again after removal of the fee (107). Also in a study that investigated participation in mammography screening among low-income women, a higher participation rate was observed in women who were assigned a voucher for a free mammogram compared to those who had to pay the fee. The main reason for non-participation among women without a voucher was costs, while it was transportation for those with a voucher (112). In most counties in Sweden, there has been a fee for cervical screening (189), which could have been an obstacle for cervical cancer attendance. As of January 2018, cervical cancer screening is free-of-charge throughout the country (190), which is important for reducing cost-related barriers to screening.

Previous studies from Sweden have reported that screening participation is lower among younger women, women who are single, immigrant women and women who are outside the labor force (108, 109). A large recent population-based case-control study on 600,000 Swedish women, showed a strong association between socioeconomic status and screening participation. Important factors associated with lower screening participation were low



disposable family income, low education and not cohabiting (110). This suggests that there are socioeconomic inequalities in access to screening which need to be targeted.

A meta-analysis on qualitative research addressed how women's experiences and perceptions influenced screening uptake. Two major themes were identified: questioning the relevance and the value of participation and screening posing a threat or previous negative experiences (111). Some of the women in our study also expressed the latter reason, which is something to address further.

### **6.3.3 Methodological considerations and validity**

The strength of this study is that it provides important estimates of on non-medical direct and indirect costs related to cervical cancer screening. This is something that has not always been accounted for in previous economic analyses. These findings can be used in future cost-effectiveness evaluations. Also, this study offers important insights on associations of HPV knowledge and screening compliance. A methodological strength is our high-quality data, as the non-participation rate was negligible, and there was neither any missing data. The questionnaire used in this study was neither formally validated; however, the cost section was based on recommendations from a working group on how to inquire patient-reported costs and the knowledge questions based on previously used questionnaires for this area, as described in detail in the methods section. Also, the Cronbach's alpha coefficient showed acceptable results regarding internal reliability. The questionnaire was also piloted prior to the study without any findings of misunderstandings or misclassifications.

The limitations of this study include that the study was performed in a county without fee for cervical cancer screening, which restricted us from examining its potential influence on screening compliance. On the other hand, this fee has now been removed throughout the country (190), as stated above, and is not as important today. Also, our cost estimates for women living in this capital region, might not be transferable to other settings in Sweden with longer geographical distances to screening clinics and less possibility to use transportation by walking or cycling (i.e. modes without any additional costs). In our material, almost 40% of women used transportations without costs. Additionally, longer distances results in increased travel costs but also longer time off work, and higher costs due to production loss related to this. On the other hand, women who had a companion to screening and those in need of child care in order to attend screening incurred higher costs. It is unclear if there are differences throughout the country with respect to these issues.

Another important limitation is that this study only included women attending cervical cancer screening and we do not have information on correlates of screening compliance for women who never attend screening. Further, when we compared our study women to the general population, there was a significant underrepresentation of women of low socioeconomic status, suggesting a possible selection bias. On the other hand, we know from the studies stated above that women of low socioeconomic status are underrepresented in screening (110), and our study population might be fairly representative. Our findings that cost and having difficulties in taking time off work could be barriers to screening could still be applicable to other socioeconomic groups as well.

## **6.4 STUDY IV**

### **6.4.1 Main findings and interpretations**

This study found that one third of the cone biopsies was incomplete at one or more margins, or of uncertain margin status. Median follow-up time was 10 years and almost nine out of ten women were followed 6 to 16 years. We found that 12% of the women developed residual or recurrent high-grade dysplasia or worse during follow-up and there were a higher proportion of involved margins in the recurrence group compared to the non-recurrence group.

Our study showed that women with involved margins had statistically significantly worse outcomes compared to women with negative margins even after adjustment for comorbidity, age at surgery, the severity of the lesion, treatment modality and previous conization procedures. The risk for residual or recurrent CIN2+ was almost 3-fold higher among women with involved endocervical margins, involved margins and uncertain if endocervical or vaginal, and especially when both margins were involved compared to women with negative margins. Also, women with history of previous conization procedure and comorbidities were at higher risk of recurrent CIN2+.

Few women were HPV-tested at any time during follow up, which was expected as the women in our study were treated before the introduction of HR-HPV test as a follow-up test-of-cure. In women with an HPV test taken, margin status remained statistically significantly associated with increased risk of recurrent or residual disease. Also in women who were positive for HR-HPV during follow-up, there was a tendency of an increased risk for recurrent CIN2+ among women with involved margins, although it did not reach formal statistical significance, probably due to power limitations.

### **6.4.2 Comparisons with other studies**

Residual or recurrent disease was detected in 12% of women, in our study which was higher than overall findings of 7 to 9% in two meta-analyses (97, 98), even though the first analysis also included any grade of post-treatment disease (98). The higher proportion in our study might be explained by our long follow-up time, enabling us to detect late recurrence. One of the meta-analyses included studies with a lower limit of 18 months mean follow-up (97), and the other had no lower limit (the shortest with 3 months follow-up) (98). Another causal factor to the high recurrence rate in our study could be that 31% of the margins were involved, which was higher than the 23% observed in the meta-analyses (range 3-60) (97, 98). According to the European Federation of Colposcopy (EFC), a complete excision rate of above 80% is considered to be an important quality indicator (217). Both meta-analyses showed a 4-6 fold higher risk of recurrent CIN2+ in women with involved margins compared to negative margins (97, 98), corroborating our findings on a positive association between incomplete excision and recurrence.

It has been shown that HR-HPV test is more accurate to use during follow-up than margin status, with higher sensitivity (91% vs. 56%) and equal specificity (both 84%) to detect recurrent high-grade disease (97). The Swedish national cervical cancer guidelines from 2017 recommend co-testing (cytology + HPV-DNA test) as a test-of-cure during follow-up. It is advised against re-treatment on the basis of positive margins alone (47). We strongly agree with this. Data from meta-analyses have shown only one out of five women with involved margins develop residual or recurrent disease (97, 98). However, we found consistently worse outcomes in women with involved endocervical margins, in women where it was

uncertain whether or not the endocervical margin was involved, and especially in women where both margins were involved compared to those with negative margins. Similar findings have been reported in other studies (218). Also, the recent meta-analysis confirmed women with involved endocervical margins to be a high-risk group for recurrent disease compared to women with negative margins (97).

It could be that women with involved endocervical margins or involvement of both margins, who are HR-HPV persistent during follow-up, should be handled more rapidly. This would be particularly important if the woman is infected with one of the more potent HR-HPV types (HPV 16/18) (55). The use of non-stratified margin status in combination with follow-up HR-HPV has not been shown to be superior in the meta-analysis (97). However, we did not find any association with residual/recurrent disease when the vaginal margin was involved alone. Nor did the meta-analyses (97, 218). In contrast, a study that only combined endocervical margin status with follow-up HPV test reported a positive predictive value of 94% and negative predictive value of 96% to predict residual or recurrent disease (219). Such combination should thus be further investigated.

#### **6.4.3 Methodological considerations and validity**

A major strength of this study was the long follow-up time, to be able to evaluate the impact of margin status and risk of residual or recurrent disease over time. Another important strength was the complete coverage of the Swedish National Cervical Screening Registry, providing reliable data on recurrent high-grade dysplasia on all included women, as previously described in the methods section. However, the registry provides only limited data on recurrence outside the cervix (VAIN, vaginal cancer, or recurrence to the pelvis or other location), which still could be a recurrent CIN. Hence, this was included as a residual or recurrent disease case when data on such was found in the medical record but the true recurrence rate could still be slightly higher. Still, this should not have influenced our findings in any major way. Neither do we have information on re-treatments or subsequent hysterectomies that occurred outside Karolinska Hospital.

A major limitation was that we only had information on HPV status on one out of five women, of which less than 10% were HR-HPV positive at any time during follow-up. It could be that the women who were HPV-tested had a higher baseline risk for recurrent disease than women who were not tested. Also, women who were diagnosed and treated for residual/recurrent disease could have a longer follow-up time with an increased chance of HPV test being taken. Due to the risk of introducing bias when adjusting for this variable, we decided not to include it in the Cox regression analysis. On the other hand, we performed a separate Cox regression in which we stratified for HPV status to further evaluate the potential of HPV as a potential confounder. It was however found that the association with involved margins remained and almost reached statistical significance also for HR-HPV positive women, despite the rather small sample size. This may speak against confounding by HPV on our findings of a positive association between margin status and recurrent disease.

## **7 CONCLUSIONS**

### **7.1 STUDY I**

This study found a very high HPV vaccination acceptance among young Argentinean women from a non-metropolitan region, and acceptance was also high when vaccination was not free of charge. The latter is especially important, since there is no subsidized catch-up vaccination for this age group in Argentina. This holds great potential for disease prevention among Argentinean women at high risk of cervical cancer. However, women who received welfare were less willing to pay for vaccination, which suggests that cost could be a barrier and may lead to socioeconomic inequalities in uptake. Also, women who did not perceive vaccination as safe in general were less willing to accept vaccination. Educational campaigns ensuring the safety of vaccines, as well as clarifying other misconceptions are needed.

### **7.2 STUDY II**

In conclusion, this study has shown a high HPV vaccination acceptance for daughters among mothers from a non-metropolitan part of Argentina. This is of major importance for a country with insufficient cervical cancer screening coverage and a high burden of cervical cancer. It was also shown that acceptance of vaccination decreased when out of pocket payment was needed. Further, being gainfully employed and having a higher disposable household income was significantly associated with acceptance of vaccination if it was not free. Taken together, these results suggest that cost could be an obstacle to catch-up vaccination. Also, women with prior awareness of cervical cancer were more willing to pay for HPV vaccination, indicating the importance of improving awareness of HPV and its related diseases.

### **7.3 STUDY III**

The results of this investigation show that the estimated total time and travel costs and direct non-medical cost for women attending cervical cancer screening in Sweden was substantial and might be an obstacle to screening and may affect the overall cost-effectiveness. It was also found that nearly half of the women did not attend screening within one year from their invitation, of which half cited difficulties in taking time off work. The most important correlates of higher screening compliance were not needing to take time off work, not having a companion and being of higher HPV knowledge. Increased flexibility by extended opening hours and improved general knowledge of HPV may facilitate screening compliance.

### **7.4 STUDY IV**

This research has shown that 12% of the women treated for precancerous lesions were diagnosed with residual or recurrent disease after a median of 10 years follow-up. Cox regression analyses revealed that women with involved margins had significantly worse long-term outcomes compared to women with negative margins. The risk was almost 3-fold higher among women with involved endocervical margins, involved margins and uncertain as to whether these were endocervical or ectocervical, and especially when both margins were involved. These findings suggest that stratified margin status may contribute to the safety of the follow-up surveillance-

## 8 FUTURE DIRECTIVES

There is still very little research on HPV vaccination acceptance from Argentina, both regarding parental vaccination acceptance and acceptance among adults. To the best of my knowledge, only one additional study has been published on this topic since our research was conducted. There is a continued lack of large-scale nationwide studies. Also, information on the actual uptake of HPV vaccination from Argentina is limited and of uncertain quality. It is of great importance to achieve high HPV vaccination uptake in a country like Argentina, with high cervical cancer burden and suboptimal screening. However, it has been reported that full-dose vaccination coverage was only 50% in the 2000 birth cohort (118), and even lower local figures have been presented, with as 2-dose uptake of only 25% in the Chaco province based on data from 2012 (215). If national coverage is as low as these figures suggest, it is vital to understand why and to target these issues. Further research should explore this. Findings from our studies suggest that educational campaigns ensuring the safety of vaccines, as well as clarifying other misconceptions are needed. However, this might be more complex than it seems. There was a mass media campaign in connection with the introduction of the HPV vaccines in Argentina but it was stopped due to criticism (185). Therefore, strategies for developing and implementing educational campaigns for this particular setting are needed. Our findings also suggest that cost could likely pose a barrier to catch-up vaccination, as this is to be self-financed in Argentina. Thus, this can lead to socioeconomic inequalities in uptake among the catch-up population and may increase the already existing inequalities in cervical cancer burden throughout the country. This is something that decision makers should address. On a more promising note, Argentina has recently introduced HPV based screening (220). It will be very interesting to follow this process and to see if it can improve the country's screening.

Also, our finding that cost can be a barrier to participation in cervical cancer screening in Sweden is important to continue to explore. A large recent Swedish study has shown that women of low socioeconomic status are underrepresented in screening (110). It is thus important that the screening organization facilitates the participation of all women in Sweden, including all socioeconomic groups. Unskilled workers may have less possibility to take time off work compared to non-manual workers. One possibility to address this could be through increased opening hours in order to facilitate attendance, irrespective of the type of work. Future research should address this. Also, it is important to explore reasons for bringing a companion to screening, and to investigate if these women also are the same as those who describe previous negative experiences of screening. A promising field is the use of HPV self-sampling for non-attendees of screening in order to improve screening uptake. It will be of great interest to follow the research development in this area and to see if it can be used among non-attendees also on a nationwide basis.

Last but not least, our findings showed promising results on the use of sub-stratified margin status to assess the risk of recurrent disease among women previously treated for cervical cancer. The possibility of using sub-divided margin in combination with HR-HPV to identify individuals in need of re-treatment should be addressed. The clinical implication and the accuracy of such combination strategies should be further investigated.

## 9 POPULÄRVETENSKAPLIG SAMMANFATTNING

Livmoderhalscancer är en sjukdom som till stor del går att förebygga med screening och förebyggande vaccination mot humant papillomvirus (HPV). Trots det är det globalt sett den fjärde vanligaste cancerformen hos kvinnor och drabbar årligen en halv miljon kvinnor, med en kvarts miljon relaterade dödsfall. Till skillnad från andra cancerformer inträffar ungefär hälften av alla nya fall bland kvinnor yngre än 50 år. Förekomsten av livmoderhalscancer uppvisar stora geografiska skillnader, och ungefär 85 % av nya fall och dödsfall sker i låginkomstländer. Det finns två huvudsakliga typer av livmoderhalscancer; skivepitelcancer och adenocarcinom. Dessa utgår från två av de celltyper som finns i livmodertappen, och den vanligaste är skivepitelcancer som utgör strax över 80 % av alla fall. Livmoderhalscancer orsakas av HPV som är den vanligaste sexuellt överförbara infektionen globalt. HPV kan även orsaka cancer som drabbar munhåla, halsmandlar, ändtarmsöppningen, slida, vulva och penis. Det finns 13 stycken så kallade hög-risk HPV-typer som kan orsaka livmoderhalscancer: HPV 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59 och 68. Av dessa är HPV 16 och 18 de vanligaste och orsakar ungefär 70 % av all livmoderhalscancer. Det är dessa två typer som ingår i de två vacciner som används i de nationella vaccinationsprogrammen runt om i världen (Cervarix<sup>®</sup> och Gardasil<sup>®</sup>). Sedan 2014 finns det ytterligare ett HPV-vaccin som täcker totalt nio HPV-typer (6, 11, 16, 18, 31, 33, 45, 52, och 58, Gardasil 9<sup>®</sup>). Detta vaccin skyddar mot ungefär 90 % av all livmoderhalscancer och finns nu även tillgängligt i Sverige. Hos de flesta läker HPV-infektionen ut spontant, men i ungefär 10 % kvarstår infektionen, och kan över tid utvecklas till livmoderhalscancer. Screening för livmoderhalscancer syftar till att upptäcka förstadier till livmoderhalscancer, så kallade cellförändringar, och behandla dessa innan cancer utvecklas. Alla cellförändringar utvecklas inte till cancer utan kan gå tillbaka spontant, men möjligheten till utläkning minskar med ökande grad av cellförändringar. Screening har funnits i Sverige sedan 1960-talet och har minskat förekomsten av livmoderhalscancer med ungefär två tredjedelar. Den nedgång man förut såg i incidensen av livmoderhalscancer i Sverige har stagnerat de senaste åren, och cancerformen ökar för närvarande igen. Kvinnor som inte deltar i screeningen och kvinnor som tidigare har haft cellförändringar har visat sig ha en ökad risk för livmoderhalscancer. I Argentina är livmoderhalscancer fortfarande den andra vanligaste cancerformen hos kvinnor och dödligheten har legat oförändrad de senaste 30 åren. Landet har så kallad opportunistisk screening, vilket innebär att kvinnor inte kallas regelbundet till screening såsom i Sverige, utan istället tas cellprov om kvinnor söker gynekolog av annan anledning. Detta är inte en effektiv metod, vilket är en stor bidragande faktor till att sjukdomsburden fortfarande är hög i landet. Sedan 2011 har Argentina gratis vaccination mot livmoderhalscancer för 11-åriga flickor. HPV-vaccination förutspås ha allra störst inverkan i länder som Argentina, med en hög förekomst av livmoderhalscancer och en inte fullt så välfungerande screening.

Det övergripande syftet med denna avhandling är att bidra till att förbättra förebyggandet av livmoderhalscancer, både i länder med hög och låg sjukdomsburden. Den relativa betydelsen av de olika förebyggande metoderna skiljer sig åt i olika delar av världen. Därför var avsikten att studera viljan att vaccinera sig mot HPV i Argentina, att identifiera vad som utgör barriärer och möjliggöra ett effektivare screeningdeltagande i Sverige, samt att undersöka långtidsrisken att återinsjukna med cellförändringar hos kvinnor som tidigare genomgått behandling för cellförändringar i Sverige utifrån om det fanns cellförändringar i operationsmaterialets resektionsrand.

I både den första och andra delstudien undersökte vi vad som påverkade viljan att vaccinera sig mot HPV hos kvinnor i Mendoza-provinsen i Argentina. I den första studien undersöktes viljan att vaccinera sig mot HPV hos 174 stycken unga kvinnor i åldrarna 18-30 år med hjälp

av ett strukturerat intervjuformulär. Nästan alla kvinnorna sa sig vara villiga att vaccinera sig mot HPV och tre fjärdedelar sa sig vara villiga att betala för vaccinationen. Kvinnor med generell tilltro till vaccin var mer villiga att vaccinera sig mot HPV, och kvinnor som inte fick socialbidrag var mer villiga att betala för vaccination. I den andra studien undersöktes viljan att låta vaccinera sina döttrar, hos 180 mödrar till flickor i åldrarna 9-15 år genom frågeformulär. Nio av tio mödrar var villiga att vaccinera sina döttrar om vaccination var gratis och 60 % sa sig även vara villiga att betala för vaccination. Kvinnor som hade hört talas om livmoderhalscancer innan studien var mer villiga att betala för HPV-vaccination, vilket betonar vikten av att öka medvetenheten om sjukdomen i samhället. Förvärvsarbetande mödrar samt kvinnor med högre disponibel hushållsinkomst var mer villiga att betala för vaccination. Detta, tillsammans med fynden i den första delstudien pekar på att höga vaccinkostnader kan utgöra ett hinder för HPV-vaccination av flickor som är äldre än 11 år och inte omfattas av vaccinationsprogrammet. Dessa individer behöver själva stå för kostnaderna, vilket kan leda till social orättvisa gällande tillgång till vaccination. Ytterligare fann studierna ett behov av att bemöta och förtydliga HPV-vaccinets säkerhet.

I den tredje delstudien efterforskade vi eventuella hinder till screeningdeltagande i Sverige. Totalt 1510 kvinnor som deltog vid den organiserade cellprovtagningen i Stockholm svarade på ett frågeformulär gällande resekostnader och andra kostnader för att delta vid screeningen, samt kunskap om livmoderhalscancer och HPV. Vi fann att genomsnittskostnaden för produktionsbortfall, resekostnader och andra utlägg för kvinnan var hög (EUR 55.6), och skulle kunna påverka deltagande i screeningen samt screeningprogrammets kostnadseffektivitet. Vidare fann vi att nästan hälften av alla kvinnor inte hade gått på screeningen inom ett år från sin kallelse, varav hälften angav svårighet att ta ledigt från arbetet. De viktigaste faktorerna som påverkade screeningdeltagandet var att inte behöva ta ledigt från arbetet, att inte ha en anhörig eller annan medföljande till besöket, och att ha högre kunskap om HPV. Kunskapen om HPV var generellt låg, men något bättre bland yngre. Ökad kunskap om HPV skulle kunna öka screeningdeltagandet. Eftersom kvinnor med sämre deltagande i screeningen till stor del uppgav svårigheter att ta ledigt från arbetet kan ett sätt att öka följsamheten vara att förändra öppettiderna till att inkludera kvällar och helger. Utöver detta skulle kostnader kunna utgöra ett hinder för deltagande. Därför är det viktigt att den organiserade screeningen möjliggör deltagande för alla kvinnor i Sverige, oavsett socioekonomisk grupp. Den nyligen borttagna avgiften för cellprovtagning i hela Sverige är ett mycket bra och välkommet exempel på detta.

I den fjärde och sista delstudien undersöktes långtidsrisken för återkomst av cellförändringar hos kvinnor som tidigare genomgått kirurgisk behandling av höggradiga cellförändringar (så kallad konisering), utifrån om det fanns cellförändringar i operationsmaterialets resektionsrand. Nästan 1000 kvinnor som behandlats på Karolinska Universitetssjukhuset i Stockholm under åren 2000-2007 följdes upp med hjälp av data från det Nationella Kvalitetsregisteret för Cervixcancerprevention. Under uppföljningstiden på upp till 16 år diagnosticerades 12 % av kvinnorna med kvarvarande eller återkomna höggradiga cellförändringar. Kvinnor som hade cellförändringar i resektionsranden hade en ökad risk för återkomst av sjukdom jämfört med kvinnor med fri resektionsrand. Risken var trefaldigt ökad om cellförändringarna fanns i resektionsranden mot livmodern, om det var oklart huruvida cellförändringarna fanns i resektionsranden mot livmodern eller mot slidan, och i synnerhet om cellförändringarna fanns i bägge resektionsränderna. Däremot fann vi ingen ökad risk om cellförändringarna bara fanns i resektionsranden mot slidan. Dessa fynd indikerar att riskbedömningen av patienter efter behandling skulle kunna förbättras om hänsyn togs till de kvarvarande cellförändringarnas lokalisation. Framtida studier bör undersöka möjligheten att kombinera fynden från resektionsranden med nuvarande praxis.

Sammanfattningsvis har denna avhandling bidraget med information om vad som kan utgöra hinder för HPV-vaccination och deltagande i screening mot livmoderhalscancer och gett förslag på möjliga förbättringsområden för att öka täckningsgraden. Att bemöta och förtydliga HPV-vaccinets säkerhet och att öka kunskapen om HPV i befolkningen skulle kunna vara viktiga åtgärder. Våra fynd indikerar att kostnad kan vara ett hinder både för vaccinationstäckning i Argentina samt vid deltagande vid screening i Sverige, vilket vårdens beslutsfattare bör ta i beaktande. Slutligen har denna avhandling visat att kvinnor med kvarvarande cellförändringar i resektionsranden efter kirurgisk behandling har en ökad risk för återkomst av cellförändringar, och att olika risker kunde ses beroende på var de kvarvarande cellförändringarna fanns. Framtida forskning bör se över möjligheten till att kombinera fynden från resektionsranden med gällande uppföljningsstrategier. Dessutom finns ett stort behov av uppföljning efter införandet av HPV-vaccination i Argentina.



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